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Estimates of nuclear DNA content in red algal lineages

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Abstract

Background and aims The red algae are an evolutionarily ancient group of predominantly marine organisms with an estimated 6000 species. Consensus higher-level molecular phylogenies support a basal split between the unicellular Cyanidiophytina and morphologically diverse Rhodophytina, the later subphylum containing most red algal species. The Rhodophytina is divided into six classes, of which five represent early diverging lineages of generally uninucleate species, whose evolutionary relationships are poorly resolved. The remaining species compose the large (27 currently recognized orders), morphologically diverse and typically multinucleate Florideophyceae. Nuclear DNA content estimates have been published for <1 % of the described red algae. The present investigation summarizes the state of our knowledge and expands our coverage of DNA content information from 196 isolates of red algae.

Methodology The DNA-localizing fluorochrome DAPI (4′,6-diamidino-2-phenylindole) and RBC (chicken erythrocytes) standards were used to estimate 2C values with static microspectrophotometry.

Principal results Nuclear DNA contents are reported for 196 isolates of red algae, almost doubling the number of estimates available for these organisms. Present results also confirm the reported DNA content range of 0.1–2.8 pg, with species of Ceramiales, Nemaliales and Palmariales containing apparently polyploid genomes with 2C = 2.8, 2.3 and 2.8 pg, respectively.

Conclusions Early diverging red algal lineages are characterized by relatively small 2C DNA contents while a wide range of 2C values is found within the derived Florideophyceae. An overall correlation between phylogenetic placement and 2C DNA content is not apparent; however, genome size data are available for only a small portion of red algae. Current data do support polyploidy and aneuploidy as pervasive features of red algal genome evolution.

Introduction

The Second Plant Genome Size Workshop and Discussion Meeting (hosted by the Royal Botanic Gardens, Kew, 8–12 September 2003) identified major gaps (systematic, regional and plant type) in our knowledge of plant DNA amounts (Bennett and Leitch 2005*a*, *b*). It was noted that no database was available for algae. This major gap was addressed with a compilation of genome size estimates for 247 species of macroscopic marine algae, including data for 95 isolates and species of red algae (Kapraun 2005). These data have been incorporated into a database of plant genome

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sizes (Kapraun *et al.* 2004) compiled and hosted by the Royal Botanic Gardens (RBG) Kew web page (http://data.kew.org/cvalues/). A subsequent investigation of green algae resulted in an expansion of coverage and characterization of the ancestral land plant flagellate genome (Kapraun 2007). More recently, efforts to expand coverage of DNA contents in brown algae were published (Phillips *et al.* 2011). This final report in the series summarizes nuclear DNA content data for red algae, both from our continuing investigations and from the literature. Results are updated at http://people.uncw.edu/kapraund/DNA (see links to 'Rhodophyta'). The present paper provides nuclear genome size estimates for 77 additional isolates of red algae and compiles all available data (196 species/isolates) into a single resource [see Additional Information]. Of this new list, 40 resulted from our ongoing research. Unicellular microalgae and freshwater red algae, which were previously under-represented (Kapraun 2005), are emphasized here.

Inclusion of published nuclear DNA content data for red algae in the present report was sometimes problematic. The Second Plant Genome Size Workshop and Discussion Meeting (Bennett and Leitch 2005*b*) identified 'best practice' methodology for nuclear genome size estimation in plant tissues. Virtually none of the published genome size data for algae resulted from investigations adhering to all of the best practice recommendations, primarily because measurement of the relatively small algal nuclear genomes requires standard species different from those specified as appropriate for vascular plants (Doležel *et al.* 1998; Kapraun 2005). A comprehensive discussion on standard species and methods is included in the section 'Notes on Appendix I'.

The red algae (Rhodophyta) are predominantly marine organisms with >700 genera and 6000 species described in 38 orders (Guiry and Guiry 2011). The Rhodophyta are characterized by unstacked thylakoids in plastids, plastids containing the accessory pigments phycoerythrin, phycocyanin and allophycocyanin arranged in phycobilisomes, the lack of plastid endoplasmic reticulum, the presence of pit connections between cells in filamentous genera and the absence of flagellated cells in the life history (Woelkerling 1990). There are a variety of current higher-level classification schemes for red algae (Saunders and Hommersand 2004; Yoon *et al.* 2006; Guiry and Guiry 2011). Molecular analyses (Oliveira and Bhattacharya 2000; Yoon *et al.* 2002*a*, *b*, 2006) and organelle ultrastructure (Pueschel 1989; Scott and Broadwater 1990) support an early divergence for the Cyanidiales, which are resolved as a sister group to other red algae and classified as a separate subphylum (Cyanidiophytina). The remaining Rhodophyta are divided into six classes that are grouped as a single subphyllum (Yoon *et al.* 2006) or multiple subphyla (Saunders and Hommersand 2004; Guiry and Guiry 2011). Five of these classes, Porphyridiophyceae, Stylonematophyceae, Compsopogonophyceae, Rhodellophyceae and Bangiophyceae, are early diverging lineages of generally uninucleate species, whose evolutionary relationships are poorly resolved (Yoon *et al.* 2006; Verbruggen *et al.* 2010). These five classes represent about 1 % of the total number of described red algal species. The remaining species are typically multinucleate and classified within the Florideophyceae, a large class of 27 currently recognized orders falling within five subclasses represented by clades that terminate long, basally positioned branches in molecular phylogenies with specific synapomorphic pit plug characteristics (Saunders and Bailey 1997; Le Gall and Saunders 2007; Verbruggen *et al.* 2010).

The traditional view that the Acrochaetiales are the most primitive and the Ceramiales are the most highly derived of the florideophycidean red algal orders (Kylin 1956; Dixon 1973) is not supported by molecular data (e.g. Le Gall and Saunders 2007; Verbruggen *et al.* 2010). A more complex phylogenetic model is emerging for red algae, characterized by ancient lineages often terminating in modern radiations (Yoon *et al.* 2006; Le Gall and Saunders 2007; Verbruggen *et al.* 2010).

New availability of both a DNA C-values database (Kapraun *et al.* 2004) and consensus higher-level phylogenies has opened the way for determining evolutionary trends in DNA amounts for other red algae (Kapraun 2005). The present static microspectrophotometric investigation of additional species of red algae was initiated to determine the extent of nuclear DNA content variation, to identify any correlation between genome size and phylogenetic relationships, and to corroborate an alternation of haploid and diploid nuclear DNA contents in gametophyte and sporophyte tissue, respectively, of selected species.

Materials and methods

Species collection data and/or source of cultures for newly reported data are summarized at http://people.uncw.edu/kapraund/DNA (see links to 'Rhodophyta'). Algal material was fixed in Carnoy's solution (Kapraun 2005) and stored in 70 % ethanol at 4 °C. Selected specimens were rehydrated in water and softened in 5 % w/v ethylenediaminetetraacetic acid (Goff and Coleman 1986, 1987, 1990) for 12–48 h. Algal specimens were transferred to coverslips treated with subbing solution, and then air dried and stained with DAPI (4′,6-diamidino-2-phenylindole) (0.5 μg mL^{-1}) (Sigma

Chemical Co., St Louis, MO, USA) as previously described (Goff and Coleman 1986, 1987, 1990; Kapraun and Nguyen 1994). Nuclear DNA contents were based on estimates from both microspectrophotometry and image analysis. Microspectrophotometry with DAPI followed procedures published previously (Kapraun and Nguyen 1994; Kapraun *et al.* 2007) using a protocol modified after Goff and Coleman (1990). Nuclear DNA content estimates based on image analysis of DAPI-stained specimens followed a procedure modified from Kapraun and Dunwoody (2002) and Choi *et al.* (2004), using a Cooled CCD Miramax RTE 782-Y high-performance digital camera placed on a Leica DMRB fluorescence microscope and analysed with MetaMorph software (Molecular Devices, Toronto, Ontario, Canada) (Gómez *et al.* 2010). For a comprehensive review of the theory and practice of DNA quantification by densitometry, see Hardie *et al.* (2002).

Nuclear DNA contents of algal specimens were estimated by comparing their I_f values with those of chicken erythrocytes (RBC) (Kapraun 1994; Kapraun and Dunwoody 2002). The rationale for accepting 2C DNA = 2.4 pg as the standard is included in 'Notes on Appendix I, Section (f)' [see Additional Information]. 4′,6-Diamidino-2-phenylindole binds by a non-intercalative mechanism to adenine- and thymine-rich regions of DNA that contain at least four A-T base pairs (Portugal and Waring 1988). Consequently, chicken erythrocytes can be used directly as standards for determining amounts of DNA only when the A–T contents of both standard and experimental DNA are equivalent (Coleman *et al.* 1981). Chicken has a nuclear DNA base composition of 42–43 mol% G + C (Marmur and Doty 1962). Limited published data for the Rhodophyta indicate values in the range of 35–42 mol% G + C (Freshwater *et al.* 1990; Le Gall *et al.* 1993; Kapraun *et al.* 1993*a*, 1996). Members of the Rhodophyta investigated in this study are assumed to have a similar range of base pair compositions, and the linearity is accepted between DAPI–DNA binding in both RBC and algal samples (Le Gall *et al.* 1993).

The Rhodophyta include taxa with some or all of their cells being multinucleate or endopolyploid (Kapraun and Nguyen 1994; Kapraun 2005) as well as taxa that exhibit a nuclear 'incremental size decrease associated with a cascading down of DNA contents' (Kapraun 1994). Methodologies were developed for specific specimens to permit assignment of C level and interpretation of I_f data. However, assignment of estimated nuclear DNA contents to specific C-values in the present study is presumptive in that no karyological investigations were conducted on the algal samples used for nuclear DNA content estimates.

Previously unpublished nuclear DNA content data in Appendix I are indicated by (°). Supplementary materials and methods, information for collection locations and data for number of algal nuclei examined in each sample and estimates of nuclear genome size (pg) ± SD are available at http://people.uncw.edu/kapraun/DNA. Nuclear DNA content data are also incorporated into a database of plant genome sizes (Kapraun 2005; Gregory *et al.* 2007) hosted by the RBG Kew web page (http://data.kew.org/cvalues/).

Results and discussion

DNA content by group

Red algal DNA contents are presented following the two subphylum classification schemes of Yoon *et al.* (2006) and florideophycean classification of Saunders and Hommersand (2004) and Le Gall and Saunders (2007).

Cyanidiophyceae The Cyanidiophyceae are small, unicellular, anciently diverged red algae (Barbier *et al.* 2005; Coppin *et al.* 2005) whose evolutionary relationships remain a subject of controversy (Garbary *et al.* 1980; Gabrielson *et al.* 1985; Seckbach 1999; Müller *et al.* 2001*a*; Yoon *et al.* 2006). Molecular data support their placement as sister to the other Rhodophyta (Saunders and Hommersand 2004; Yoon *et al.* 2006), but the unique attributes of these organisms (Coppin *et al.* 2005) provide a sense that the cyanidiophytes are as distinct from other red algae as are phyla in the plant and animal kingdoms relative to one another (Saunders and Hommersand 2004; Yoon *et al.* 2004, 2006). Putative synapomorphies of the cyanidiophytes include a blue-green colour resulting from the presence of α-chlorophyll and C-phycoerythrin, complete lack of the red phycoerythrins (De Luca *et al.* 1978) and an ability to inhabit hot, acidic waters (acidothermophilic) (Seckbach 1999). The cyanidiophytes are reported to have the smallest known genomes of any phototrophic eukaryotes (Matsuzaki *et al.* 2004). Pulse-field gel electrophoresis (PFGE) and Feulgen microspectrophotometry with *Saccharomyces cerevisiae* Meyen *ex* Reess standard have yielded 1C nuclear genome size estimates of 10–16 Mbp (Suzuki *et al.* 1992; Maleszka 1993; Matsuzaki *et al.* 2004; Barbier *et al.* 2005) and $1.35–2.25 \times 10^{-2}$ pg (Muravenko *et al.* 2001) in eight isolates and species of these extremophile algae [see Additional Information]. The size of the nuclear genome in *Cyanidioschyzon* reported in the last decade has doubled as a result of progress in measuring techniques (Matsuzaki *et al.* 2004). It is assumed that these recent values are more accurate. Consequently, earlier nuclear genome size estimates listed here should be treated with caution.

Porphyridiophyceae, Stylonematophyceae, Compsopogonophyceae and Rhodellophyceae These classes were traditionally included with the Bangiophyceae in a group variously classified as a subphylum or subclass. Early molecular studies indicated that this was a polyphyletic grouping of distinct lineages (e.g. Freshwater *et al.* 1994; Müller *et al.* 2001*b*), and recent studies have assigned these lineages to separate classes (e.g. Saunders and Hommersand 2004; Yoon *et al.* 2006). The Porphyridiophyceae consists solely of unicellular forms including *Porphyridium* and *Flintiella*, while unicellular and pseudofilamentous taxa such as *Rhodosorus*, *Stylonema* and *Goniotrichopsis* make up the Stylonematophyceae (West *et al.* 2005; Yoon *et al.* 2006). The Compsopogonophyceae includes taxa of various morphologies that have been treated as separate families (Rintoul *et al.* 1999) or orders (Silva *et al.* 1996), but which form a monophyletic lineage in most analyses (e.g. Yoon *et al.* 2006; Verbruggen *et al.* 2010). The Rhodellophyceae is another group of primarily unicells such as *Dixoniella* and *Rhodella*. Relationships among these lineages are similar in the concatenated multilocus DNA sequence analyses of Yoon *et al.* (2006) and Verbruggen *et al.* (2010), but these relationships are poorly supported.

Few nuclear DNA content estimates are available for members of these early diverging lineages, and the current values are all relatively small (Fig. 1). Two species exemplifying these low values are *Compsopogon caeruleus* (Balbis ex C. Agardh) Montagne (Compsopogonales, Compsopogonophyceae) with a 2C DNA content of 0.2 pg and a reported chromosome complement of $1n = 7 \pm 1$ (Nichols 1964), and *Erythrotrichia carnea* (Dillwyn) J. Agardh (Erythropeltidales, Compsopogonophyceae) with a 2C DNA content of 0.7 pg. These data are consistent with a basal (ancestral) red algal genome characterized both by small genome sizes and small chromosome complements. In addition, the small range of the nuclear DNA content values in these early diverging lineages (0.1–0.7; Fig. 1) suggests that the long evolutionary separation of these lineages was not accompanied by substantial changes in DNA content [see Additional Information].

Bangiophyceae This class, as presently understood is monophyletic and includes 15 currently recognized extant genera (some still unnamed) (Sutherland *et al.* 2011). The chief characteristic used to separate the familiar genera *Bangia* and *Porphyra*, e.g. filament vs. blade, lacks taxonomic significance as these morphologies arose independently several times throughout the evolutionary diversification of the Bangiales (Oliveira *et al.* 1995; Müller *et al.* 2001*a*, *b*; Broom *et al.* 2004; Jones *et al.* 2004; Milstein and de Oliveira 2005). Molecular data do not support the distinction of *Bangia* and *Porphyra* as monophyletic genera, and analyses of these data have resulted in the transfer of a majority of species previously placed in *Porphyra*, including species of commercial value, to new genera (e.g. Nelson *et al.* 2006; Sutherland *et al.* 2011). DNA sequence analyses also suggest that the simple morphology of these organisms obscures significant levels of genetic diversity, including the presence of morphologically cryptic species (Klein *et al.* 2003; Müller *et al.* 2003; Nelson *et al.* 2003; Sutherland *et al.* 2011). Recently, two species of *Porphyra* were transferred to two new genera, *Pyrophyllon* and *Chlidophyllon*, which are included in the Erythropeltidales (Compsopogonophyceae) (Nelson *et al.* 2003). In addition to clarifying taxonomic classifications and identifying cryptic species, molecular data have been useful in recognizing the conspecific status of some widely distributed species (Broom *et al.* 2002; Neefus and Brodie 2009), including *Pyropia suborbiculata* (Kjellm.) J.E. Sutherl., H.G. Choi, M.S. Hwang *et* W.A. Nelson and *Pyropia elongata* (Kylin) Neefus *et* J. Brodie, which we investigated previously as *Porphyra carolinensis* Coll *et* J. Cox and *Porphyra rosengurtii* Coll *et* J. Cox, respectively (Kapraun and Freshwater 1987; Kapraun *et al.* 1991; Kapraun 2005). Transfer of the *Pyropia spiralis* (E.C. Oliveira *et* Coll) M.C. Oliveira, D. Milstein *et* E.C. Oliveira variety previously studied as *Porphyra spiralis* var. *amplifolia* E.C. Oliveira *et* Coll is needed after the generic reclassification of Sutherland *et al.* (2011) and is effected here:

Pyropia spiralis (E.C. Oliveira *et* Coll) M.C. Oliveira, D. Milstein *et* E.C. Oliveira var. *amplifolia* (E.C. Oliveira *et* Coll) Freshwater *et* Kapraun comb. nov.

Basionym: *Porphyra spiralis* var. *amplifolia* E.C. Oliveira and Coll (1975: p. 196, Figs 3, 10).

In the eight isolates of *Bangia*, *Porphyra* and *Pyropia* investigated, neither estimates of 2C nuclear DNA contents, which range from 0.6 to 1.2 pg, nor published chromosome complements, which range from $1n = 3–5$, appear to be genus specific (Kapraun 2005). The representation of these species and isolates in current phylogenetic studies (e.g. Sutherland *et al.* 2011) is insufficient to determine whether there is any relationship between nuclear DNA content and evolutionary patterns of the various Bangiophyceae lineages.

Florideophyceae—Nemaliophycidae The Florideophyceae includes five currently recognized subclasses (Saunders and Hommersand 2004; Le Gall and Saunders 2007). DNA content estimates are available for representatives of the Nemaliophycidae, Corallinophycidae and

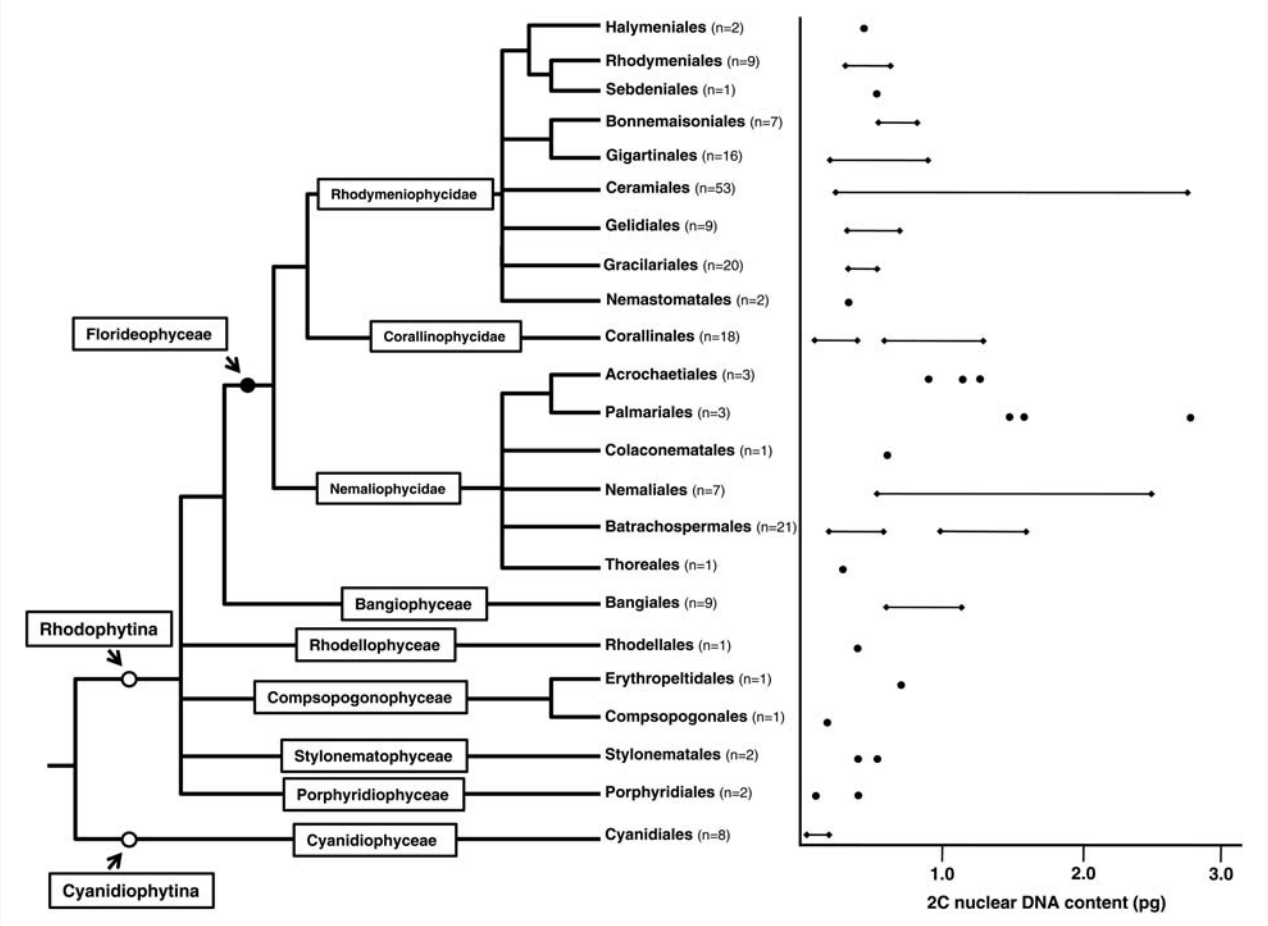

Fig. 1 Estimated 2C nuclear DNA contents superimposed on a consensus red algal phylogeny. Phylogeny based on the analyses of Yoon *et al.* (2006), Le Gall and Saunders (2007) and Verbruggen *et al.* (2010) with unsupported nodes in these analyses collapsed to polytomies. Branch labels follow the two-subphylum classification of Yoon *et al.* (2006) and florideophycean classification of Saunders and Hommersand (2004) and Le Gall and Saunders (2007). Dots represent individual DNA content estimates; lines represent the range of values for multiple species.

Rhodymeniophycidae. The remaining two subclasses, Ahnfeltiophycidae and Hildenbrandiophycidae, while evolutionarily distinct, include only a small number of species. Genome size estimates are available for six of the nine currently recognized Nemaliophycidae orders, and the group is characterized by a nuclear 2C DNA content range of 0.2–2.8 pg (Fig. 1).

Acrochaetiales, Palmariales, Colaconematales, Nemaliales. Molecular systematic investigations have resolved a close relationship among members of the Acrochaetiales, Palmariales, Colaconematales and Nemaliales (Saunders *et al.* 1995; Harper and Saunders 2002; Huisman *et al.* 2004), which are considered to represent early lineages of florideophytes. The transfer of *Rhodothamniella floridula* (Dillwyn) Feldmann from the Acrochaetiales (Saunders *et al.* 1995) and segregation of the Colaconematales (Harper and Saunders 2002) has resulted in a monophyletic Acrochaetiales that is sister to the Palmariales (e.g. Clayden and Saunders 2010). Although the relationships of the Colaconematales and Nemaliales are poorly resolved in large red algal phylogenies (Le Gall and Saunders 2007; Verbruggen *et al.* 2010), the more specific analysis of Clayden and Saunders (2010) indicates a sister relationship between these orders.

Nuclear DNA content data have been published for only one species of the Colaconematales, *Colaconema daviesii*, with 2C DNA = 0.6 pg, and are limited to two species of *Audouinella* in the Acrochaetiales [see Additional Information]. Data available for species of the Nemaliales suggest that this order is characterized by one of the

largest ranges of DNA contents (2C = 0.5–2.5 pg) of any of the florideophytes (Fig. 1). The Palmariales, as presently delimited (Clayden and Saunders 2010), includes three genera for which nuclear DNA content estimates are available: *Devaleraea* (Guiry 1982), *Palmaria* (Guiry 1974) and *Rhodothamniella* (Saunders *et al.* 1995). A 2C range of 1.5–2.8 pg gives the Palmariales the largest mean nuclear DNA size in the florideophytes.

Batrachospermales/Thoreales. Until recently, freshwater red algae belonging to the genera *Thorea* and *Nemalionopsis* were included in the order Batrachospermales (Kumano 2002). Small subunit ribosomal DNA and *rbc*L sequence analyses indicate that the Thoreaceae has been misclassified in the Batrachospermales and should be placed in its own order, the Thoreales (Müller *et al.* 2002). Species in this order are characterized by having freshwater representatives with multiaxial gametophytes, a uniaxial chantransia stage and pit plugs with two cap layers, the outer one of which is usually plate like. Nuclear DNA content estimate data are available for only one species in this order, *Thorea riekei*, with 2C = 0.28 pg [see Additional Information]. The Batrachospermales is distinguished from other freshwater rhodophyte orders based on a heterotrichous life history phase, lack of tetraspore production, and a two-layered pit plug, the outer layer of which is domed (Vis *et al.* 1998).

The Batrachospermales and the Thoreales are of particular interest as they are exclusively freshwater (Vis and Sheath 1997) in the Nemaliophycidae clade, which includes several additional orders that are primarily or at least partially freshwater: Acrochaetiales, Balbiniales, Balliales (Harper and Saunders 2001; Müller *et al.* 2001*a*, *b*; Le Gall and Saunders 2007). As members of the distantly related Compsopogonales (Compsopogonophyceae) are primarily freshwater as well (Sheath 1984; Müller *et al.* 2002), it is likely that adaptation to freshwater habitats involved multiple, independent events in the evolution of red algae.

The genus *Batrachospermum* appears to be polyphyletic, comprising many morphologically similar but distantly related taxa (e.g. Chiasson *et al.* 2007; Kapraun *et al.* 2007). Species of *Batrachospermum*, *Sirodotia* and *Tuomeya* (Batrachospermaceae) investigated in the present study have 2C nuclear DNA contents of about 0.2–0.6 pg, while species of *Lemanea* and *Paralemanea* (Lemaneaceae) have noticeably larger 2C genome sizes of 1.0–1.6 pg [see Additional Information]. Results of this study suggest a possible correlation between polyploidy and the expression of the *Batrachospermum* or *Lemanea* morphological phenotypes.

Published karyological studies for Batrachospermaceae indicate that most species have chromosome numbers in the range of $1n = 3–5$ or 10–12, while Lemaneaceae species have chromosome complements of $1n = 15–20$ (Kapraun *et al.* 2007). Both the larger genome sizes and chromosome complements in *Lemanea* and *Paralemanea* are consistent with polyploidy events in their common ancestry.

A unique pattern of somatic meiosis has been described in members of this order associated with development of haploid gametophytes from vegetative branches of the microscopic, diploid sporophyte phase (Necchi and Carmona 2002). The sporophyte phase has been described variously as '*Chantransia*' (Chiasson *et al.* 2005), *Audouinella* (Necchi and Zucchi 1997) and, possibly, *Balliopsis* (Saunders and Necchi 2002). Support for this life history comes from both cytological (von Stosch and Theil 1979; Necchi 1987) and microspectrophotometry (Sheath *et al.* 1994, 1996) investigations. In the present study, DAPI and microspectrophotometry demonstrated in isolates of three species (*Batrachospermum gelatinosum*, *Batrachospermum vagum* and *Lemanea torulosa*) I_f (fluorescence) levels in 2C nuclei in presumptive gametophytes that closely approximate 50 % of the 4C values in presumptive sporophytes.

Florideophyceae—Corallinophycidae Past molecular systematic investigations resolved the Corallinales as a lineage within the larger group of taxa that share the presence of pit plugs with two cap layers and were classified as the Nemaliophycidae (Saunders and Bailey 1997; Harper and Saunders 2002; Saunders and Hommersand 2004). The recent multigene study of Le Gall and Saunders (2007) demonstrated that the Corallinales and Rhodogorgonales represented a separate evolutionary lineage from the Nemaliophycidae, and established the Corallinophycidae. The analyses of Verbruggen *et al.* (2010) supported this classification and the inclusion of the Sporolithales in this subclass as suggested by Le Gall and Saunders (2007).

Nuclear DNA content data are only available from species in the Corallinales where 2C DNA contents range from 0.1 to 1.3 pg (Fig. 1) [see Additional Information]. Coralline algae can be divided into two types: geniculate (with alternating calcified internodes and uncalcified nodes) and non-geniculate (which usually grow as crusts) (e.g. Woelkerling *et al.* 1993). Recently, molecular studies demonstrated that genicula are non-homologous structures that evolved independently in several families (Bailey and Chapman 1996, 1998). When DNA content data are superimposed on this molecular phylogeny, it becomes apparent that geniculate clades are represented by species with larger

nuclear genomes (0.6–1.3 pg) while non-geniculate clades contain species with relatively small nuclear genomes (0.1–1.0 pg) (Kapraun 2005). Analysis of additional species will be required to determine whether these observations reflect a sampling artefact.

Florideophyceae—Rhodymeniophycidae Ordinal classification within the Rhodymeniophycidae continues to be refined, in large part as a result of distinct evolutionary lineages being recognized within the large, polyphyletic Gigartinales (e.g. Withall and Saunders 2006). The subclass currently includes 12 orders (Saunders and Hommersand 2004; Guiry and Guiry 2011), with DNA content estimates available for species in nine of these (Fig. 1). While the overall 2C DNA content range of 0.2–2.8 pg is relatively wide, five of the orders (Gelidiales, Gigartinales, Gracilariales, Halymeniales and Rhodymeniales) have particularly narrow ranges of DNA contents [see Additional Information].

Gelidiales. The relatively narrow range of small DNA content values but substantial range of chromosome numbers (Kapraun and Bailey 1989; Freshwater 1993; Kapraun *et al.* 1993*b*, 1994), and the absence of a correlation between nuclear genome size and chromosome number suggest a significant role of aneuploidy in Gelidialean evolution (Kapraun and Dunwoody 2002). Analyses of DNA sequence data from a variety of loci have resulted in a consistent molecular phylogeny for the Gelidiales (e.g. Freshwater and Bailey 1998; Shimada *et al.* 1999; Thomas and Freshwater 2001; Tronchin and Freshwater 2007). This well-circumscribed order includes only a handful of genera, but is particularly species rich (e.g. Millar and Freshwater 2005), and it would be very interesting to explore the possible role of aneuploidy in their evolution by obtaining additional chromosome and genome size data for representative species.

Bonnemaisoniales. This order was separated from the Nemaliales on the basis of their then known alternation of generations (Feldmann and Feldmann 1942). It is now known that this life history pattern lacks taxonomic significance as some Nemaliales are heteromorphic and some Bonnemaisoniales are isomorphic (Womersley 1996). For example, *Bonnemaisonia asparagoides* (Woodward) C. Agardh is monoecious and has a direct life history with no tetrasporophyte, while *Bonnemaisonia clavata* G. Hamel is dioecious and has an alternation of heteromorphic generations with '*Hymenoclonium serpens*' representing the tetrasporophyte (Salvador Soler *et al.* 2008). The Bonnemaisoniales are currently recognized at the ordinal level on the morphological basis of their apical development pattern and direct development of the gonimoblast. Ultrastructural details of pit plugs and caps (Pueschel 1989) and plastids (Chihara and Yoshizaki 1972), as well as molecular studies (e.g. Saunders *et al.* 2004; Le Gall and Saunders 2007), appear to support retention of this order.

In a recent study (N. Salvador Soler, University of Barcelona, Barcelona, Spain, unpubl. res.) and the current study, nuclear DNA content data for '*Falkenbergia rufolanosa*', the diploid sporophyte phase of the heteromorphic species *Asparagopsis armata*, and for the isomorphic species *Delisea plumosa* and *Ptilonia willana*, suggest 2C values for members of this order of 0.5–0.6 pg. Nuclear DNA content data for both phases of the heteromorphic species in the Bonnemaisoniales are needed to confirm that ploidy level shifts (2*n*/4*n*) are associated with the gametophyte and sporophyte phases, respectively.

Ceramiales. The Ceramiales is the largest red algal order, with close to 400 genera and 1500 species described (Kraft and Woelkerling 1990; Schneider and Wynne 2007; Wynne and Schneider 2010). Genome size data are available for fewer than 2 % of these species [see Additional Information]. Members of this order have both the largest DNA contents and the greatest range of DNA content values (0.26–2.8 pg). Past molecular systematics investigations indicate that the traditional ceramialean families, Dasyaceae, Delesseriaceae and Rhodomelaceae, evolved from a paraphyletic Ceramiaceae (de Jong *et al.* 1998; Phillips 2000; Lin *et al.* 2001; Choi *et al.* 2002; Zuccarello *et al.* 2002; Barros-Barreto *et al.* 2006). Choi *et al.* (2008) proposed a new taxonomy for the Ceramiales that split the paraphyletic traditional Ceramiaceae into the Ceramiaceae *sensu stricto* and three new families, Callithamniaceae, Spyridiaceae and Wrangeliaceae (Fig. 2). When nuclear DNA content data are superimposed on a consensus molecular phylogeny for the order, each family is seen to have at least one (ancestral?) species with a 2C DNA content of <1.0 pg as well as species with elevated (polyploid?) DNA contents (Fig. 2). The simplest explanation is that polyploidy, characterized by even number multiple increases in chromosome complements as well as increase in nuclear genome size, accompanied speciation in each of these lineages. A strong correlation between chromosome complements and nuclear genome size in many Ceramiales investigated is consistent with this explanation, although analysis of

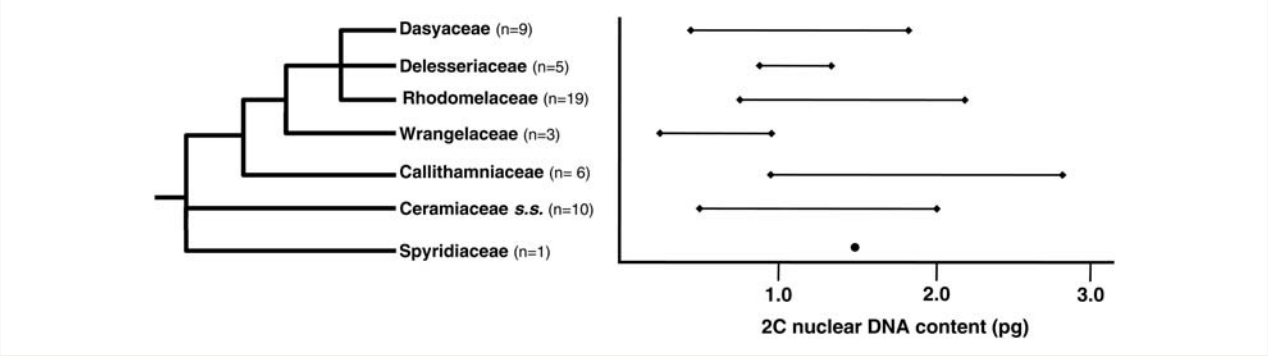

Fig. 2 Estimated 2C nuclear DNA contents superimposed on a family phylogeny for the Ceramiales. Phylogeny based on analyses of Choi *et al.* (2008) with unsupported nodes collapsed to polytomies. Dots represent individual DNA content estimates; lines represent the range of values for multiple species.

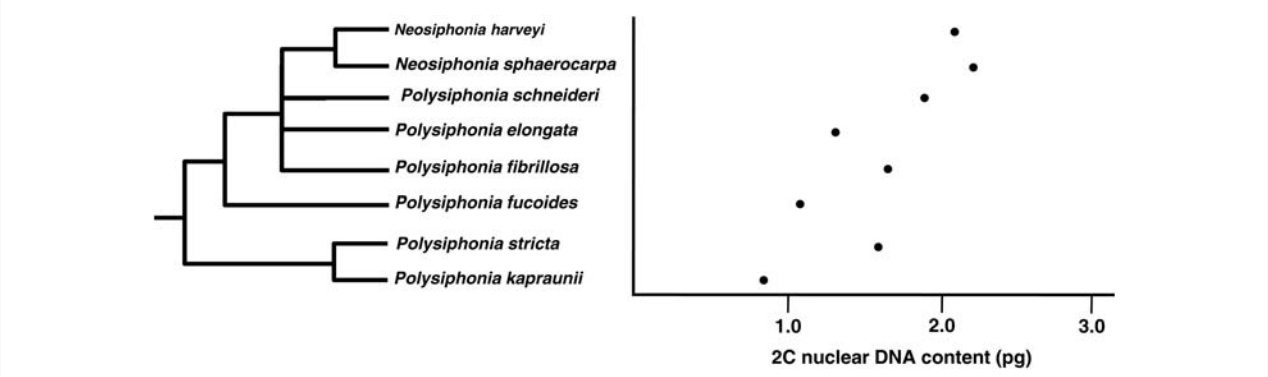

Fig. 3 Estimated 2C nuclear DNA contents superimposed on a phylogeny of *Neosiphonia* and *Polysiphonia* species. Phylogeny based on the analyses of Mamoozadeh and Freshwater (2011).

additional species will be required to eliminate the possibility that our observations reflect sampling error. Conspicuous exceptions include *Acanthophora spicifera* with $2n = 64$ and $2C = 1.1$ pg, and *Antithamnion villosum* with $2n = 48$ and $2C = 2.0$ pg. Clearly, in some genera, polyploidy events were followed by chromosome reorganization, including fission/fusion processes ultimately resulting in aneuploidy as described for species of the Rhodomelaceae genus *Polysiphonia* (Kapraun 1993*a*).

Molecular systematic investigations have demonstrated the paraphyly of *Polysiphonia sensu lato* (e.g. Choi and Kim 2001; Stuercke and Freshwater 2010; Mamoozadeh and Freshwater 2011), especially in relation to the recently described genus *Neosiphonia* (Kim and Lee 1999). The currently available data are insufficient to explore any relationships of genome size and species evolution (Fig. 3). However, now that a more accurate understanding of phylogenetic relationships is emerging, it would be of interest to determine whether nuclear genome sizes and chromosome complements have diagnostic value in delimiting the monophyletic species groups being revealed within *Polysiphonia sensu lato*.

Recent data suggest that Ceramiales are an ancient lineage relative to other Rhodymeniophycidae (Le Gall and Saunders 2007; Verbruggen *et al.* 2010), yet on average they have larger nuclear genome contents than most of the taxa that are believed to have diverged after them. Unless an assumption is made that the other taxa in the Rhodymeniophycidae lineage have experienced nuclear genome size decrease, an explanation is required to account for the larger genome sizes in the Ceramiales.

Although the existence of mechanisms for decreasing DNA amounts have been proposed (Wendel *et al.* 2002), it is more probable that polyploidy and transposable

element amplification will result in genome size increase through time (Bennetzen 2002), ultimately resulting in genomic 'obesity' (Bennetzen and Kellogg 1997). Since the Ceramiales are arguably the oldest members of the Rhodymeniophycidae lineage, they would have accumulated the largest genomes and may have been subject to a predictable genomic expansion. Although data are severely limited, there appears to be a correlation between antiquity of these red algal lineages and their mean nuclear DNA contents.

Gigartinales. The Gigartinales is a large and diverse order (Fredericq *et al*. 1996; Hommersand *et al*. 1999; Tai *et al*. 2001; Saunders *et al*. 2004) including commercially important carrageenophytes such as *Eucheuma*, *Kappaphycus* and *Chondrus* (Craigie 1990; Kapraun 1999). Present results confirm previous studies (Kapraun *et al*. 1992; López-Bautista and Kapraun 1995; Kapraun and López-Bautista 1997), suggesting that members of this order are characterized by a wide range of chromosome numbers ($2n = 10–70$) and a narrow range of small nuclear DNA contents (2C = 0.2–0.9 pg) [see Additional Information]. The genome size (1C) of *Chondrus crispus* was estimated as 150 Mbp using flow cytometry of haploid nuclei (Le Gall *et al*. 1993), but recent complete sequencing of this genome indicates a size of only 105 Mbp (Collén 2011) concordant with previous estimates using static microspectrophotometry (Kapraun 2005). This relatively small size and the species' economic importance made *Chondrus* an ideal candidate among carrageenophytes for genome sequencing.

Halymeniales. The Halymeniales is a relatively large order of 270+ species classified in 26 currently recognized genera (Guiry and Guiry 2011). Currently, 2C DNA content data are only available for two species, *Grateloupia filicina* (Lamouroux) C. Agardh and *Halymenia floridana* J. Agardh, with both having identical values (Fig. 1) [see Additional Information].

Nemastomatales and Sebdeniales. Recent studies have reinstated the Nemastomatales and established the Sebdeniales for species previously part of the Gigartinales (Saunders and Kraft 2002; Withall and Saunders 2006). Although the orders are represented by relatively few species, molecular and morphological analyses reveal additional diversity (e.g. Schneider *et al*. 2006). Currently, estimates of 2C DNA content are only available for two *Predaea* (Nemastomatales) and one *Sebdenia* (Sebdeniales) species (Fig. 1) [see Additional Information].

Gracilariales. This order includes relatively few genera, but some of them, e.g., *Gracilaria*, are species rich (Fredericq and Hommersand 1990). No new nuclear DNA content estimates are available for this order, but previous data indicate that nuclear genome sizes are small (0.3–0.5 pg) (Kapraun 1993*b*, 2005). The Gracilariales, unlike the Gelidiales, is noted for genome size constancy, with all species of *Gracilaria* investigated having identical 2C DNA contents of 0.4 pg and chromosome complements of $2n = 48$ (Kapraun and Dutcher 1991; Kapraun 1993*a*). Species of the closely related *Gracilariopsis* (Bird *et al*. 1994; Bellorin *et al*. 2002; Gurgel *et al*. 2003) exhibit some variation in both 2C DNA contents (0.3–0.5 pg) and $2n$ chromosome complements with values of $2n = 48$ and 64 reported.

Rhodymeniales. Nuclear DNA content estimates from previous (Kapraun 2005) and present studies are now available for nine species representing three families of the Rhodymeniales (Saunders *et al*. 1999) [see Additional Information]. This order, along with the Gelidiales and Gracilariales, has both a narrow range and a small mean nuclear genome size (2C = 0.3–0.6 pg).

Range of DNA contents

The size of the red algal genomes reported here and previously (Kapraun 2005) is best appreciated when compared with the minimum amount of DNA estimated for specifying the mRNA sequences required for angiosperm development. Specifically, the genomes of *Genlisea margaretae* Hutchinson and *Arabidopsis thaliana* (L.) Heynhold, with 2C = 126 and 314 Mb, respectively, (Riechmann *et al*. 2000; Bennett *et al*. 2003; Greilhuber *et al*. 2006), are among the smallest found in angiosperms (Bennett and Smith 1976), but still have 1.5–2 times the estimated 15 000 genes per haploid genome required for development (Flavell, 1980). Similarly, the genome of the green alga *Volvox carteri* F. Stein, with 138 Mbp, has an estimated coding potential for 14 500 proteins (Prochnik *et al*. 2010). Even the smallest rhodophyte genome reported (e.g. 1C = 98 Mb in *Compsopogon coerulus*), with its probable genomic redundancy (Kapraun 2005, 2007), has the genic capacity for morphologically complex development.

Polyploidy

Polyploidy has been reported widely in the Rhodophyta (Cole 1990; Kapraun 2005), especially in the Ceramiales, which have both the largest nuclear genomes and the highest chromosome numbers (Kapraun 1993*a*, 2005; Kapraun and Dunwoody 2002). For a recent review of

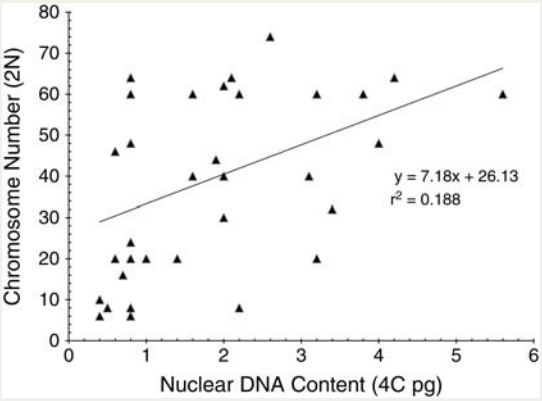

Fig. 4 Comparison of 4C nuclear DNA contents and 2*n* chromosome complements for 33 species of Florideophyceae. DNA content values from the present study and previously published data (Kapraun 2005; Kapraun *et al.* 2007; Salvador Soler *et al.* 2009), and 2*n* chromosome complements from Cole (1990) and Kapraun (2005).

concepts associated with adaptations and genetic variability associated with hybridization and polyploidy in algae, see Coyer *et al.* (2006). Comparison of 2*n* chromosome numbers and 2C nuclear DNA contents shows a poor relationship ($r^2 = 0.188$, Fig. 4), consistent with a high occurrence of aneuploidy, i.e. chromosomal fusion and/or fission events (Kapraun *et al.* 1993*b*; Kapraun 2005). Present results support previous suggestions (Kapraun 1989) that polyploidy and aneuploidy are pervasive features of red algal genomics. The extent of both species-level and intraplant ploidy-level variation (including endopolyploidy) remains to be determined (Goff and Coleman 1986, 1987), but represents an exciting area for future research.

Correlation between DNA content and phylogenetic placement

Although no correlation is apparent between phylogenetic placement and genome size, groups considered to be basal (Cyanidiophytina, Porphyridiophyceae, Stylonematophyceae, Compsopogonophyceae, Rhodellophyceae) generally have genome sizes $\leq$0.5 pg, while derived groups (Bangiophyceae, Florideophyceae) generally have genome sizes $\geq$0.5 pg, with values up to 2.8 pg reported. DNA contents may be diagnostic, synapomorphies in both the Corallinales and Batrachospermales. In the Corallinales (Kapraun 2005), geniculate clades are represented by species with larger nuclear genomes (0.6–1.3 pg) while non-geniculate clades contain species with relatively small nuclear genomes (0.1–1.0 pg), the overlap in these ranges is a result of single outlier species. Similarly, in the Batrachospermales, species of *Batrachospermum*, *Sirodotia* and *Tuomeya* have 2C nuclear DNA contents of 0.2–0.6 pg while species of *Lemanea* and *Paralemanea* have noticeably larger 2C genome sizes of 1.0–1.6 pg (Kapraun *et al.* 2007) [see Additional Information]. More definitive trends may be revealed as data for nuclear genome size and our understanding of red algal evolutionary relationships increase.

Correlation between DNA content and habitat

It is likely that adaptation to freshwater habitats involved multiple, independent events in the evolution of red algae. In the present study, no correlation between nuclear genome size and adaptation to freshwater habitats is apparent in the Compsopogonales, Thoreales and Batrachospermales.

Correlation between nuclear genome size and reproductive parameters

In a previous investigation of the relationship of nuclear genome size to reproductive cell parameters in the Rhodophyta (Kapraun and Dunwoody 2002), three general trends regarding carpospore production were noted: (i) increase in genome size was positively correlated with increase in carpospore volume; (ii) species with larger genome sizes produced fewer carpospores; and (iii) species that produced larger carpospores produce fewer carpospores. Members of the Ceramiales, with their larger genome sizes, typically produce fewer, but larger carpospores and generally behave as predicted in a *K*-selection model. In contrast, members of the Gelidiales, Gigartinales and Gracilariales, with their smaller genome sizes, typically produce large numbers of small carpospores as predicted in an *r*-selected model (Kapraun and Dunwoody 2002). The conspicuous limitation of this ecological model is that the Ceramiales generally produce small, structurally simple, short-lived plants (associated with *r*-selection), while the other orders generally produce large, structurally complex, long-lived plants (associated with *K*-selection).

Characteristics of an ancestral red algal genome

In the present study, cyanidophytes represent the earliest diverging red algal lineage and have reported genome sizes of 1C = 0.02–0.1 pg (1C = 10–55 Mbp) and 2*n* chromosome numbers between 4 and 20 [see Additional Information]. These genomic characteristics recommend this group for further investigations that could possibly help characterize the nuclear genome in unicellular organisms prior to the transition to multicellularity seen in other red algae. Among the basal Rhodophytina, members of the Compsopogonophyceae,

Porphyridiophyceae and Stylonematophyceae may represent appropriate candidates for investigations of nuclear genomes in extant, basal red algae. For example, *Compsopogon caeruleus* has a 2C DNA content of 0.25 pg (1C = 98 Mb) and a reported chromosome complement of $1n = 7 \pm 1$ (Nichols 1964). Small genome size and chromosome complements have been reported in *Porphyridium aerugineum* Geitler ($n = 2$) and *P. purpureum* (Bory de Saint-Vincent) K.M. Drew & R. Ross [as *P. cruentum* (S.F. Gray) Nägeli] ($n = 2$; 2C DNA content = 0.1 pg) (Sommerfeld and Nichols 1970), although it remains unclear whether these represent haploid or diploid values.

Candidates for genomic studies

DNA C-value remains a key character in biology, biodiversity and molecular investigations as genome size has many important practical implications (Bennett *et al.* 2000). Genome size directly influences the cost and difficulty of sequencing projects, and was a primary consideration in choosing subjects for early whole-genome analyses (Gregory 2001, 2005), including those of algae where small DNA content (haploid genomes ~100 Mbp) has been a major criterion (Peters *et al.* 2004; Waaland *et al.* 2004). Despite major improvements in sequencing cost and efficiency provided by current next-generation sequencing technology, genome size is still a consideration for coverage and *de novo* assembly. Many red algal species have haploid genomes in the range of 127–300 Mbp [see Additional Information], and the present study provides a list of target species with small genome sizes for whole-genome sequencing studies. Many of these species (e.g. Gelidiales and Gracilariales) are also amenable to culture and are of significant ecological and/or commercial importance (López-Bautista and Kapraun 1995; Kapraun and López-Bautista 1997; Kapraun 1999).

Conclusions and forward look

Early diverging red algal lineages are characterized by relatively small 2C DNA contents while a wide range of 2C values is found within the derived Florideophyceae. An overall correlation between phylogenetic placement and 2C DNA content is not apparent; however, genome size data are available for only a small portion of red algae. Current data do support polyploidy and aneuploidy as pervasive features of red algal genome evolution.

Red algae that warrant further investigation include the Nemaliales, Acrochaetiales and Colaconematales. Phylogenetic analyses indicate that these three orders are part of early diverging florideophycean lineages (e.g. Le Gall and Saunders 2007), are widely distributed and contain many genera that are species rich (Saunders *et al.* 1995; Harper and Saunders 1998), yet published information about their genome sizes is very limited. It would be of interest to determine whether the relatively wide range of DNA contents found in the Nemaliales occurs in these other related orders.

Another group of red algae that warrant attention is the Ceramiales, especially the Rhodomelaceae, which may include more species than all other red algae combined. Continuing molecular phylogenetic investigations provide us with evolutionary schemes (e.g. Martin-Lescanne *et al.* 2010; Mamoozadeh and Freshwater 2011) upon which genome size data can be superimposed to reveal the extent that speciation was accompanied by nuclear transformations.

Additional information

The following additional information is available in the online version of this article –

File 1. Appendix I—Chromosome numbers and nuclear DNA content estimates in isolates and species of red algae.

File 2. Notes on Appendix I.

File 3. Numbered references for chromosome complements and DNA content estimates in the Rhodophyta cited in Appendix I.

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Contributions by the authors

All microspectrophotometry was conducted by D.K. (University of North Carolina Wilmington, USA). D.W.F. (Center for Marine Science, University of North Carolina Wilmington, USA) and D.K. contributed algal cultures and/or field-collected materials, and prepared the manuscript for publication.

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Conflict of interest statement

None declared.

References

Bailey JC, **Chapman RL**. **1996**. Evolutionary relationships among coralline red algae (Corallinaceae, Rhodophyta) inferred from 18S rRNA gene sequence analysis. In: Chaudhary BR, Agrawal SB, eds. *Cytology, genetics and molecular biology of algae*. Amsterdam: SPB Academic Publishing, 363–376.

Bailey JC, **Chapman RL**. **1998**. A phylogenetic study of the Corallinales (Rhodophyta) based on nuclear small-subunit rRNA gene sequences. *Journal of Phycology* **34**: 692–705.

Barbier G, **Oesterhelt C**, **Larson MD**, **Halgren RG**, **Wilkerson C**, **Garavito RM**, **Benning C**, **Weber APM**. **2005**. Comparative genomics of two closely related unicellular thermo-acidophilic red algae, *Galdieria sulphuraria* and *Cyanidioschyzon merolae*, reveals the molecular basis of the metabolic flexibility of *Galdieria sulphuraria* and significant differences in carbohydrate metabolism of both algae. *Plant Physiology* **137**: 460–474.

Barros-Barreto MBB, **McIvor L**, **Maggs CA**, **Gerreira PCG**. **2006**. Molecular systematics of *Ceramium* and *Centroceras* (Ceramiaceae, Rhodophyta) from Brazil. *Journal of Phycology* **42**: 905–921.

Bellorin AM, **Oliveira MC**, **Oliveira EC**. **2002**. Phylogeny and systematics of the marine algal family Gracilariaceae (Gracilariales, Rhodophyta) based on small subunit rDNA and its sequences of Atlantic and Pacific species. *Journal of Phycology* **38**: 551–563.

Bennett MD, **Leitch IJ**. **2005*a***. Nuclear DNA amounts in angiosperms: progress, problems and prospects. *Annals of Botany* **95**: 45–90.

Bennett MD, **Leitch IJ**. **2005*b***. Plant gnome size research: a field in focus. *Annals of Botany* **95**: 1–6.

Bennett MD, **Smith JB**. **1976**. Nuclear DNA amounts in angiosperms. *Philosophical Transactions of the Royal Society of London, Series B* **274**: 227–274.

Bennett MD, **Bhandol P**, **Leitch IJ**. **2000**. Nuclear DNA amounts in angiosperms and their modern uses—807 new estimates. *Annals of Botany* **86**: 859–909.

Bennett MD, **Leitch IJ**, **Price HJ**, **Johnston JS**. **2003**. Comparisons with *Caenorhabditis* (~100 Mb) and *Drosophila* (~175 Mb) using flow cytometry show genome size in *Arabidopsis* to be ~157 Mb and thus ~25 % larger than the *Arabidopsis* Genome Initiative estimate of ~125 Mb is not ~125 Mb but approaches *Drosophila* (~175 Mb). *Annals of Botany* **91**: 547–557.

Bennetzen JL. **2002**. Mechanisms and rates of genome expansion and contraction in flowering plants. *Genetica* **115**: 29–36.

Bennetzen JL, **Kellogg EA**. **1997**. Do plants have a one-way ticket to genomic obesity? *The Plant Cell* **9**: 1509–1514.

Bird CJ, **Ragan MA**, **Critchley AT**, **Rice EL**, **Gutell RR**. **1994**. Molecular relationships among the Gracilariaceae (Rhodophyta): further observations on some undetermined species. *European Journal of Phycology* **29**: 195–202.

Broom JE, **Nelson WA**, **Yarish C**, **Jones WA**, **Agular Rosas R**, **Agular Rosas LE**. **2002**. A reassessment of the taxonomic status of *Porphyra suborbiculata*, *Porphyra carolinensis* and *Porphyra lilliputiana* (Bangiales, Rhodophyta) based on molecular and morphological data. *European Journal of Phycology* **37**: 227–235.

Broom JES, **Farr TJ**, **Nelson WA**. **2004**. Phylogeny of the *Bangia* flora of New Zealand suggests a southern origin for *Porphyra* and *Bangia* (Bangiales, Rhodophyta). *Molecular Phylogenetics and Evolution* **31**: 1197–1207.

Chiasson WB, **Sabo NJ**, **Vis ML**. **2005**. Affinities of freshwater putative chantransia stages (Rhodophyta) from molecular and morphological data. *Phycologia* **44**: 163–168.

Chiasson WB, **Johanson KG**, **Serwood AR**, **Vis ML**. **2007**. Phylogenetic affinities of the form taxon *Chantransia pygmaea* (Rhodophyta) specimens from the Hawaiian Islands. *Phycologia* **46**: 257–262.

Chihara M, **Yoshizaki M**. **1972**. Bonnemaisoniaceae: their gonimoblast development, life history and systematics. In: Abbott IA, Kurogi M, eds. *Contributions to the systematics of benthic marine algae of the North Pacific*. Kobe: Japanese Society of Phycology, 243–252.

Choi H-G, **Kim KY**. **2001**. Mixed-phase reproduction of *Dasysiphonia chejuensis* from Korea: nuclear DNA contents and environmental factors. *Algae* **16**: 437–443.

Choi H-G, **Kraft GT**, **Lee IK**, **Saunders GW**. **2002**. Phylogenetic analyses of anatomical and nuclear SSU rDNA sequence data indicate that the Dasyaceae and Delesseriaceae (Ceramiales, Rhodophyta) are polyphyletic. *European Journal of Phycology* **37**: 551–569.

Choi H-G, **Lee E-Y**, **Oh YS**, **Kim H-S**, **Lee IK**. **2004**. Nuclear DNA quantification of some Ceramialean algal spermatia by fluorescence microscopic image processing and their nuclear SSU rDNA sequences. *Algae* **19**: 79–90.

Choi H-G, **Kraft GT**, **Kim H-S**, **Guiry MD**, **Saunders GW**. **2008**. Phylogenetic relationships among lineages of the Ceramiaceae (Ceramiales, Rhodophyta) based on nuclear small subunit rDNA sequence data. *Journal of Phycology* **44**: 1033–1048.

Clayden SL, **Saunders GW**. **2010**. Recognition of *Rubrointrusa membranacea* gen. et comb. nov., *Rhodonematella subimmersa* gen. et comb. nov. (with reinterpretation of the life history) and the Meiodiscaceae fam. nov. within the Palmariales (Rhodophyta). *Phycologia* **49**: 283–300.

Cole K. **1990**. Chromosomes. In: Cole KM, Sheath RG, eds. *Biology of the red algae*. Cambridge: Cambridge University Press, 73–101.

Coleman AW, **Maguire MJ**, **Coleman JR**. **1981**. Mithramycin- and 4′ 6 diamidino-2-phenolindole (DAPI)-staining for fluorescence microspectrophotometric measurement of DNA in nuclei, plastids, and virus particles. *Journal of Histochemistry and Cytochemistry* **29**: 959–968.

Collén J. **2011**. The *Chondrus crispus* genome. In: *4th Congress of the International Society for Applied Phycology*, 19–21 June 2011, Halifax, Canada Meeting. Keynote Address, Abstract #4. **ISAP2011-halifax.org**.

Coppin A, **Varré JS**, **Lienard I**, **Dauvillée D**, **Guérardel Y**, **Soyer-Gobillard MO**, **Buléon A**, **Ball S**, **Tomayo S**. **2005**. Evolution of plant-like crystalline storage polysaccharides in the protozoan parasite *Toxoplasma gondii* argues for a red algal ancestry. *Journal of Molecular Evolution* **60**: 257–267.

Coyer JA, **Hoarau G**, **Pearson GA**, **Serrao EA**, **Stam WT**, **Olsen JL**. **2006**. Convergent adaptation to a marginal habitat by homoploid hybrids and polyploidy ecas in the seaweed genus *Fucus*. *Biology Letters* **2**: 405–408.

Craigie JS. **1990**. Chromosomes. In: Cole KM, Sheath RG, eds. *Biology of the red algae*. Cambridge: Cambridge University Press, 221–258.

De Luca P, Taddei R, Varano L. 1978. '*Cyanidioschyzon merole*': a new alga of thermal acidic environments. *Eebbia* **33**: 37–44.

Dixon P. 1973. *Biology of the Rhodophyta*. New York: Hafner Press.

Doležel J, Greilhuber J, Lucretti S, Meister A, Lysak MA, Nardi L, Obermayer R. 1998. Plant genome size estimation by flow cytometry: Inter-laboratory comparison. *Annals of Botany* **82**(Suppl. A): 17–26.

Feldmann J, Feldmann G. 1942. Recherches sur les Bonnemaisoniacées et leur alternance de générations. *Annales de Science Naturelle (Botany), Séries II* **3**: 75–175.

Flavell R. 1980. The molecular characterization and organization of plant chromosomal DNA sequences. *Annual Review of Plant Physiology* **31**: 569–596.

Fredericq S, Hommersand MH. 1990. Diagnoses and key to the genera of the Gracilariaceae (Gracilariales, Rhodophyta). *Hydrobiologia* **204/205**: 173–178.

Fredericq S, Hommersand MH, Freshwater DW. 1996. The molecular systematics of some carrageenan-containing marine red algae based on rbcL sequence analysis. *Hydrobiologia* **326/327**: 125–135.

Freshwater DW. 1993. Cytophotometric estimation of inter- and intraspecific variation in nuclear DNA content in ten taxa of the Gelidiales (Rhodophyta). *Journal of Experimental Marine Biology and Ecology* **166**: 231–239.

Freshwater DW, Bailey JC. 1998. A multigene phylogeny of the Gelidiales including nuclear large-subunit rRNA sequence data. *Journal of Applied Phycology* **10**: 229–236.

Freshwater DW, Dutcher JA, Kapraun DF, Sizemore RK. 1990. Variation in nuclear DNA base composition (mol% G + C) in three orders of marine green algae. *Hydrobiologia* **204/205**: 167–172.

Freshwater DW, Fredericq S, Butler BS, Hommersand MH, Chase MW. 1994. A gene phylogeny of the red algae (Rhodophyta) based on plastid *rbc*L. *Proceedings of the National Academy of Sciences of the USA* **91**: 7281–7285.

Gabrielson PW, Garbary DJ, Scagel RF. 1985. The nature of the ancestral red alga: inferences from a cladistic analysis. *BioSystems* **18**: 335–346.

Garbary DJ, Hansen GK, Scagel RF. 1980. A revised classification of the Bangiophyceae (Rhodophyta). *Nova Hedwigia* **33**: 145–166.

Goff LJ, Coleman AW. 1986. A novel pattern of apical cell polyploidy, sequential polyploidy reduction and intercellular nuclear transfer in the red alga *Polysiphonia*. *American Journal of Botany* **73**: 1109–1130.

Goff LJ, Coleman AW. 1987. The solution to the cytological paradox of isomorphy. *Journal of Cell Biology* **104**: 739–748.

Goff LJ, Coleman AW. 1990. DNA: microspectrofluorometric studies. In: Cole KM, Sheath RG, eds. *Biology of the red algae*. New York: Cambridge University Press, 43–72.

Gómez AG, Ribera Siguan MA, Soler NS, Rull JL, Kapraun DF. 2010. Fucales (Phaeophyceae) from Spain characterized by large-scale discontinuous nuclear DNA contents consistent with ancestral cryptopolyploidy. *Phycologia* **49**: 64–72.

Gregory TR. 2001. Coincidence, coevolution, or causation? DNA content, cell size, and the C-value enigma. *Biological Reviews* **76**: 65–101.

Gregory TR. 2005. The C-value enigma in plants and animals: a review of parallels and an appeal for partnership. *Annals of Botany* **95**: 133–146.

Gregory TR, Nicol JA, Tamm H, Kullman B, Kullman K, Leitch IJ, Murray BG, Kapraun DF, Greilhuber J, Bennett MD. 2007. Eukaryotic genome size databases. *Nucleic Acids Research* **35**: D332–338.

Greilhuber J, Borsch T, Müller K, Worberg A, Porembski S, Barthlott W. 2006. Smallest angiosperm genomes found in Lentibulariaceae with chromosomes of bacterial size. *Plant Biology* **8**: 1–8.

Guiry MD. 1974. A preliminary consideration of the taxonomic position of *Palmaria palmata* (Linnaeus) Stackhouse = *Rhodymenia palmata* (Linnaeus) Greville. *Journal of the Marine Biological Association of the United Kingdom* **54**: 509–528.

Guiry MD. 1982. *Devaleraea*, a new genus of the Palmariaceae (Rhodophyta) in the North Atlantic and North Pacific. *Journal of the Marine Biological Association of the United Kingdom* **62**: 1–13.

Guiry MD, Guiry GM. 2011. *AlgaeBase*. Galway: World-wide electronic publication, National University of Ireland. **http://www.algaebase.org**; searched July 2011.

Gurgel CFD, Liao LM, Fredericq S, Hommersand MH. 2003. Systematics of *Gracilariopsis* (Gracilariales, Rhodophyta) based on rbcL sequence analyses and morphological evidence. *Journal of Phycology* **39**: 154–171.

Hardie DC, Gregory RT, Hebert PDN. 2002. From pixels to picograms: a beginners' guide to genome quantification by Fuelgen image analysis densitometry. *Journal of Histochemistry and Cytochemistry* **50**: 735–749.

Harper JT, Saunders GW. 1998. A molecular systematic investigation of the Acrochaetiales (Florideophycidae, Rhodophyta) and related taxa based on nuclear small-subunit ribosomal DNA sequence data. *European Journal of Phycology* **33**: 221–229.

Harper JT, Saunders GW. 2001. Molecular systematics of the florideophyceae (Rhodophyta) using nuclear large and small subunit rDNA sequence data. *Journal of Phycology* **37**: 1073–1082.

Harper JT, Saunders GW. 2002. A re-classification of the Acrochaetiales based on molecular and morphological data, and establishment of the Colaconematales ord. nov. (Florideophyceae, Rhodophyta). *European Journal of Phycology* **37**: 463–476.

Hommersand MH, Fredericq S, Freshwater DW, Hughey J. 1999. Recent developments in the systematics of the Gigartinaceae (Gigartinales, Rhodophyta) based on *rbc*L sequence analysis and morphological evidence. *Phycological Research* **47**: 139–151.

Huisman JM, Harper JT, Saunders GW. 2004. Phylogenetic study of the Nemaliales (Rhodophyta) based on large-subunit ribosomal DNA sequences supports segregation of the Scinaiaceae fam. nov. and resurrection of *Dichotomaria* Lamarck. *Phycological Research* **52**: 224–234.

Jones WA, Griffin NJ, Jones DT, Nelson WA, Farr TJ, Broom JE. 2004. Phylogenetic diversity in South African *Porphyra* (Bangiales, Rhodophyta) determined by nuclear SSU sequence analyses. *European Journal of Phycology* **39**: 197–211.

de Jong YSDM, Wurff AWG van, Stam WT, Olsen JL. 1998. Studies on Dasyaceae.3. Towards a phylogeny of the Dasyaceae (Ceramiales, Rhodophyta), based on comparative *rbc*L gene sequences and morphology. *European Journal of Phycology* **33**: 187–201.

Kapraun DF. 1989. Karyological investigations of chromosome variation patterns associated with speciation in some Rhodophyta. In: George RY, Hulbert AW, eds. *Carolina Coastal Oceanography Symposium, 1987*. Wilmington, NC: National Undersea Research Progress Research Report 892, 65–76.

Kapraun DF. 1993*a*. Karyology and cytophotometric estimation of nuclear DNA content variation in *Gracilaria*, *Gracilariopsis*, and *Hydropuntia* (Gracilariales, Rhodophyta). *European Journal of Phycology* **28**: 253–260.

Kapraun DF. 1993*b*. Karyology and cytophotometric estimation of nuclear DNA variation in several species of *Polysiphonia* (Rhodophyta, Ceramiales). *Botanica Marina* **36**: 507–516.

Kapraun DF. 1994. Cytophotometric estimation of nuclear DNA contents in thirteen species of the Caulerpales (Chlorophyta). *Cryptogamic Botany* **4**: 410–418.

Kapraun DF. 1999. Red algal polysaccharide industry: economics and research status at the turn of the century. *Hydrobiologia* **399**: 7–14.

Kapraun DF. 2005. Nuclear DNA content estimates in multicellular green, red and brown algae: phylogenetic considerations. *Annals of Botany* **95**: 7–44.

Kapraun DF. 2007. Nuclear DNA content estimates in green algal lineages: Chlorophyta and Streptophyta. *Annals of Botany* **99**: 677–701.

Kapraun DF, Bailey JC. 1989. Karyology and nuclear DNA content of *Gelidium pusillum* (Gelidiales, Rhodophyta) from North Carolina, USA. *Japanese Journal of Phycology* **37**: 201–207.

Kapraun DF, Dunwoody JT. 2002. Relationship of nuclear genome size to some reproductive cell parameters in the Florideophycidae (Rhodophyta). *Phycologia* **41**: 507–516.

Kapraun DF, Dutcher JA. 1991. Cytophotometric estimation of inter- and intraspecific nuclear DNA content variation in *Gracilaria* and *Gracilariopsis* (Gracilariales, Rhodophyta). *Botanica Marina* **34**: 139–144.

Kapraun DF, Freshwater DW. 1987. Karyological studies of five species of *Porphyra* (Bangiales, Rhodophyta) from the North Atlantic and Mediterranean. *Phycologia* **26**: 82–87.

Kapraun DF, López-Bautista J. 1997. Karyology, nuclear genome quantification and characterization of the carrageenophytes *Eucheuma* and *Kappaphycus* (Gigartinales). *Journal of Applied Phycology* **8**: 465–471.

Kapraun DF, Nguyen MN. 1994. Karyology, nuclear DNA quantification and nucleus-cytoplasmic domain variations in some multinucleate green algae. *Phycologia* **33**: 42–52.

Kapraun DF, Hinson TK, Lemus AJ. 1991. Karyology and cytophotometric estimation of inter- and intraspecific nuclear DNA variation in four species of *Porphyra* (Rhodophyta). *Phycologia* **30**: 458–466.

Kapraun DF, Dutcher JA, Lopez-Bautista J. 1992. Nuclear genome characterization of the carrageenophyte *Agardhiella subulata* (Rhodophyta). *Journal of Applied Phycology* **4**: 129–137.

Kapraun DF, Dutcher JA, Freshwater DW. 1993*a*. Quantification and characterization of nuclear genomes in commercial red seaweeds: Gracilariales and Gelidiales. *Hydrobiologia* **260/261**: 679–688.

Kapraun DF, Dutcher JA, Freshwater DW. 1993*b*. DNA base composition heterogeneity in some Rhodophyta. *Cryptogamic Botany* **4**: 97–106.

Kapraun DF, Ganzon-Fortes E, Bird K, Trono G, Breden C. 1994. Karyology and agar analysis of the agarophyte *Gelidiella acerosa* (Forsskål) Feldmann et Hamel from the Philippines. *Journal of Applied Phycology* **6**: 545–550.

Kapraun DF, Lopez-Bautista J, Trono G, Bird KT. 1996. Quantification and characterization of nuclear genomes in commercial red seaweeds (Gracilariales) from the Philippines. *Journal of Applied Phycology* **8**: 125–130.

Kapraun DF, Leitch IJ, Bennett MD. 2004. Algal DNA C-values database (release 1.0, December 2004) **http://www.rgbkew.org.uk/cval/homepage.html**. (29 July 2011).

Kapraun DF, Braly KS, Freshwater DW. 2007. Nuclear DNA content variation in the freshwater red algal orders Batrachospermales and Thoreales (Florideophyceae, Nemaliophycidae). *Phycologia* **46**: 54–62.

Kim MS, Lee IK. 1999. *Neosiphonia flavimarina* gen et sp. nov. with a taxonomic reassessment of the genus *Polysiphonia* (Rhodomelaceae, Rhodophyta). *Phycological Research* **47**: 271–281.

Klein AS, Mathieson AC, Neefus CD, Cain DF, Taylor HA, Teasdale BW, West AL, Hehre DJ, Brodie J, Yarish C, Wallace AL. 2003. Identification of north-western Atlantic *Porphyra* (Bangiaceae, Bangiales) based on sequence variation in nuclear SSU and plastid *rbc*L genes. *Phycologia* **42**: 109–122.

Kraft GT, Woelkerling WJ. 1990. Rhodophyta. In: Clayton MN, King RJ, eds. *Biology of marine plants*. Melbourne: Longman Cheshire, 41–85.

Kumano S. 2002. *Freshwater red algae of the world*. Bristol: Biopress.

Kylin H. 1956. *Die Gattungen der Rhodophycee*. Lund: Gleerups.

Le Gall L, Saunders GW. 2007. A nuclear phylogeny of the Florideophyceae (Rhodophyta) inferred from combined EF2, small subunit and large subunit ribosomal DNA: Establishing the new red algal subclass Corallinophycidae. *Molecular Phylogenetics and Evolution* **43**: 1118–1130.

Le Gall Y, Brown S, Marie D, Mejjad M, Kloareg B. 1993. Quantification of nuclear DNA and G-C content in marine macroalgae by flow cytometry of isolated nuclei. *Protoplasma* **173**: 123–132.

Lin S-M, Fredericq S, Hommersand MH. 2001. Systematics of the Delesseriaceae (Ceramiales, Rhodophyta) based on large subunit rDNA and *rbc*L sequences, including the Phycodryoideae, subfam. nov. *Journal of Phycology* **37**: 881–899.

López-Bautista J, Kapraun DF. 1995. Agar analysis, nuclear genome quantification and characterization of four agarophytes (*Gracilaria*) from the Mexican Gulf Coast. *Journal of Applied Phycology* **7**: 351–357.

Maleszka R. 1993. Electrophoretic analysis of the nuclear and organellar genomes in the ultra-small alga *Cyanidioschyzon merolae*. *Current Genetics* **24**: 548–550.

Mamoozadeh NR, Freshwater DW. 2011. Taxonomic notes on Caribbean *Neosiphonia* and *Polysiphonia* (Ceramiales, Florideophyceae): five species from Florida, USA and Mexico. *Botanica Marina* **54**: 269–292.

Marmur J, Doty P. 1962. Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *Journal of Molecular Biology* **5**: 109–118.

Martin-Lescanne J, Rousseau F, De Reviers B, Payri C, Couloux A, Cruad C, Le Gall L. 2010. Phylogenetic analyses of the *Laurencia* complex (Rhodomelaceae, Ceramiales) support the recognition of five genera: *Chondrophycus*, *Laurencia*, *Osmundea*, *Palisada* and *Yuzurua* stat. nov. *European Journal of Phycology* **45**: 51–61.

Matsuzaki M, Misumi O, Shin-I T, Maruyama S, Takahara M, Miyagishima SY, Mori T, Nishida K, Yagisawa F, Nishida K, Yoshida Y, Nishimura Y, Nakao S, Kobayashi T, Momoyama Y, Higashiyama T, Minoda A, Sano M, Nomoto H, Oishi K,

Hayashi H, Ohta F, Nishizaka S, Haga S, Miura S, Morishita T, Kabeya Y, Terasawa K, Suzuki Y, Ishii Y, Asakawa S, Takano H, Ohta N, Kuroiwa H, Tanaka K, Shimizu N, Sugano S, Sato N, Nozaki H, Ogasawara N, Kohara Y, Kuroiwa T. 2004. Genome sequence of the ultrasmall unicellular red alga *Cyanidioschyzon merolae* 10D. *Nature* **428**: 653–657.

Millar AJK, Freshwater DW. 2005. Morphology and molecular phylogeny of the marine algal order Gelidiales (Rhodophyta) from New South Wales, including Lord Howe and Norfolk Islands. *Australian Systematic Botany* **18**: 215–263.

Milstein D, Oliveira MC. 2005. Molecular phylogeny of Bangiales (Rhodophyta) based on small subunit rDNA sequencing: emphasis on Brazilian *Porphyra* species. *Phycologia* **44**: 212–221.

Müller KM, Oliveira MC, Sheath RB, Bhattacharya D. 2001*a*. Ribosomal DNA phylogeny of the Bangiophycidae (Rhodophyta) and the origin of secondary plastids. *American Journal of Botany* **88**: 1390–1400.

Müller KM, Cannone JJ, Gutell RR, Sheath RG. 2001*b*. A structural and phylogenetic analysis of the group IC1 introns in the order Bangiales (Rhodophyta). *Molecular Biology and Evolution* **18**: 1654–1667.

Müller KM, Sherwood AR, Pueschel CM, Gutell RR, Sheath RG. 2002. A proposal for a new red algal order, the Thoreales. *Journal of Phycology* **38**: 807–820.

Müller KM, Cole KM, Sheath RG. 2003. Systematics of *Bangia* (Bangiales, Rhodophyta) in North America. II. Biogeographical trends in karyology: chromosome numbers and linkage with gene sequence phylogenetic trees. *Phycologia* **42**: 209–219.

Muravenko OV, Selyakh IO, Kononenko NV, Stadnichuk IN. 2001. Chromosome numbers and nuclear DNA contents in the red microalgae *Cyanidium caldarium* and three *Galdieria* species. *European Journal of Phycology* **36**: 227–232.

Necchi O. 1987. Studies on the freshwater Thodophyta of Brazil—3: *Batrachospermum brasiliense* sp. nov. from the state of Sao Paulo, southern Brazil. *Revista Brasileira de Biologia* **47**: 441–446.

Necchi O Jr, Carmona JJ. 2002. Somatic meiosis and development of the juvenile gametophyte in members of the Batrachospermales *sensu lato* (Rhodophyta). *Phycologia* **41**: 340–347.

Necchi O Jr, Zucchi MR. 1997. *Audouinella macrospora* (Acrochaetiaceae, Rhodophyta) is the 'Chantransia' stage of *Batrachospermum* (Batrachospermaceae). *Phycologia* **36**: 220–224.

Neefus CD, Brodie J. 2009. Lectotypification of *Porphyra elongata* Kylin (Bangiales, Rhodophyta) and proposed synonymy of *Porphyra rosengurttii* Coll et Cox. *Cryptogamie Algologie* **30**: 187–192.

Nelson WA, Broom JE, Farr TJ. 2003. *Pyrophyllon* and *Chlidophyllon* (Erythropeltidales, Rhodophyta): two new genera for obligate epiphytic species previously placed in *Porphyra*, and a discussion of the orders Erythropeltidales and Bangiales. *Phycologia* **42**: 308–314.

Nelson WA, Farr TJ, Broom JES. 2006. Phylogenetic relationships and generic concepts in the red order Bangiales: challenges ahead. *Phycologia* **45**: 249–259.

Nichols HW. 1964. Culture and developmental morphology of *Compsopogon coeruleus*. *American Journal of Botany* **51**: 180–188.

Oliveira Filho EC, Coll J. 1975. The genus *Porphyra* C. Ag. (Rhodophyta, Bangiales) in the American south Atlantic I. Brazilian species. *Botanica Marina* **18**: 191–197.

Oliveira MC, Bhattacharya D. 2000. Phylogeny of the Bangiophycidae (Rhodophyta) and the secondary endosymbiotic origin of algal plastids. *American Journal of Botany* **87**: 482–492.

Oliveira MC, Kurniawan J, Bird CJ, Rice EL, Murphy CA, Singh RK, Gutell RR, Ragan MA. 1995. A preliminary investigation of the order Bangiales (Bangiophycidae, Rhodophyta) based on sequences of nuclear small-subunit ribosomal RNA genes. *Phycological Research* **43**: 71–79.

Peters AF, Marie D, Scornet D, Kloareg B, Cock JM. 2004. Proposal of *Ectocarpus siliculosus* (Ectocarpales, Phaeophyceae) as a model organism for brown algal genetics and genomics. *Journal of Phycology* **40**: 1079–1088.

Phillips LE. 2000. Taxonomy of the New Zealand-endemic genus *Pleurostichidium* (Rhodomelaceae, Rhodophyta). *Journal of Phycology* **36**: 773–786.

Phillips N, Kapraun DF, Gomez Garreta A, Ribera Siguan MA, Rull JL, Salvador Soler N, Lewis R, Kawai H. 2011. Estimates of nuclear DNA content in 98 species of brown algae (Phaeophyta). *AoB PLANTS* **2011**: plr001; doi:10.1093/aobpla/plr001.

Portugal J, Waring M. 1988. Assignment of DNA binding sites for DAPI and bisbenzimide (Hoeschst 33258). Comparative footprinting study. *Biochimica et Biophysica Acta* **949**: 158–168.

Prochnik SE, Umen J, Nedeicu AM, Hallmann A, Miller SM, Nishii I, Ferris P, Kuo A, Mitros T, Fritz-Laylin LK, Hellsten U, Chapman J, Simakov O, Rensing SA, Terry A, Pangilinan J, Kapitonov V, Jurka J, Salamov A, Shapiro H, Schmutz J, Grimwood J, Lindquist E, Lucas S, Grigoriev IV, Schmitt R, Kirk D, Rokhsar DS. 2010. Genomic analysis of organismal complexity in the multicellular green alga *Volvox carteri*. *Science* **329**: 223–226.

Pueschel CM. 1989. An expanded survey of the ultrastructure of red algal pit plugs. *Journal of Phycology* **25**: 625–636.

Riechmann JL, Heard J, Martin G, Reuber L, Jiang C-Z, Keddle J, Adam L, Pineda O, Ratcliffe OJ, Samaha RR, Creelman R, Pilgrim M, Broun P, Zhang JZ, Ghandehari D, Sherman BK, Yu G-L. 2000. *Arabidopsis* transcription factors: genome-wide comparative analysis among eukaryotes. *Science* **290**: 2105–2110.

Rintoul TL, Sheath RG, Vis ML. 1999. Systematics and biogeography of the Compsopogonales (Rhodophyta) with emphasis on the freshwater families in North America. *Phycologia* **38**: 517–527.

Salvador Soler N, Gómez Garreta A, Ribera Siguan MA. 2008. Characterization of two frequently confused species, *Bonnemaisonia asparagoides* and *Bonnemaisonia clavata* (Bonnemaisoniales, Rhodophyta), on the basis of morphological and molecular evidence. *Phycologia* **47**: 177–190.

Salvador Soler N, Gómez Garreta A, Ribera Siguan MA. 2009. Somatic meiosis in the life history of *Bonnemaisonia asparagoides* and *Bonnemaisonia clavata* (Bonnemaisoniales, Rhodophyta) from the Iberian Peninsula. *European Journal of Phycology* **44**: 381–393.

Saunders GW, Bailey JC. 1997. Phylogenesis of pit-plug-associated features in the Rhodophyta: inferences from molecular systematic data. *Canadian Journal of Botany* **75**: 1436–1467.

Saunders GW, Hommersand MH. 2004. Assessing red algal supraordinal diversity and taxonomy in the context of contemporary systematic data. *American Journal of Botany* **91**: 1494–1507.

Saunders GW, Kraft GT. 2002. Two new Australian species of *Predaea* (Nemastomataceae, Rhodophyta) with taxonomic

recommendations for an emended Nemastomatales and expanded Halymeniales. *Journal of Phycology* **38**: 1245–1260.

Saunders GW, Necchi O. 2002. Nuclear rDNA sequences from *Ballia prieurii* support recognition of Balliopsis gen. nov. in the Batrachospermales. *Phycologia* **41**: 61–67.

Saunders GW, Bird CJ, Ragan MA, Rice EL. 1995. Phylogenetic relationships of species of uncertain taxonomic position within the Acrochaetiales-Palmariales complex (Rhodophyta): inferences from phenotypic and 18S rDNA sequence data. *Journal of Phycology* **31**: 601–611.

Saunders GW, Strachan I, Kraft GT. 1999. The families of the order Rhodymeniales (Rhodophyta): a molecular-systematic investigation with a description of Faucheaceae *fam. nov*. *Phycologia* **38**: 23–40.

Saunders GW, Chiovitti A, Kraft GT. 2004. Small-subunit rDNA sequences from representatives of selected families of the Gigartinales and Rhodymeniales (Rhodophyta). 3. Delineating the Gigartinales sensu stricto. *Canadian Journal of Botany* **82**: 43–74.

Schneider CW, Wynne MJ. 2007. A synoptic review of the classification of red algal genera a half century after Kylin's 'Die Gattungen der Rhodophyceen'. *Botanica Marina* **50**: 197–249.

Schneider CW, Lane CE, Saunders GW. 2006. *Crassitegula walsinghamii* (Sebdeniaceae, Halymeniales), a new red algal genus and species from Bermuda based upon morphology and SSU rDNA sequence analyses. *European Journal of Phycology* **41**: 115–224.

Scott J, Broadwater S. 1990. Cell division. In: Cole KM, Sheath RG, eds. *Biology of the red algae*. New York: Cambridge University Press, 123–145.

Seckbach J. 1999. The Cyanidiophyceae: hot spring algae. In: Seckbach J, ed. *Enigmatic microorganisms and life in extreme environments*. The Netherlands: Kluwer Academic Publishers, 425–435.

Sheath RG. 1984. The biology of the freshwater red algae. *Progress in Phycological Research* **3**: 89–157.

Sheath RG, Whittick A, Cole KM. 1994. *Rhododraparnaldia oregonica*, a new freshwater red algal genus and species intermediate between the Acrochaetiales and the Batrachospermales. *Phycologia* **33**: 1–7.

Sheath RG, Muller KM, Whittick A, Entwisle TJ. 1996. A re-examination of the morphology and reproduction of *Nothocladus lindaueri* (Batrachospermales, Rhodophyta). *Phycological Research* **44**: 1–10.

Shimada S, Horiguchi T, Masuda M. 1999. Phylogenetic affinities of genera *Acanthopeltis* and *Yatabella* (Gelidiales, Rhodophyta) inferred from molecular analyses. *Phycologia* **38**: 528–540.

Silva PC, Basson PW, Moe RL. 1996. Catalogue of the benthic marine algae of the Indian Ocean. *University of California Publications in Botany* **79**: 1–1259.

Sommerfeld MR, Nichols HW. 1970. Comparative studies in the genus *Porphyridium* Naeg. *Journal of Phycology* **6**: 67–78.

Stuercke B, Freshwater DW. 2010. Two new species of *Polysiphonia* (Ceramiales, Florideophyceae) from the Western Atlantic. *Botanica Marina* **53**: 301–311.

Sutherland JE, Lindstrom SC, Nelson WA, Brodie J, Lynch MD, Hwang MS, Choi H-G, Miyata M, Kikuchi N, Oliveira MC, Farr T, Neefus C, Mols-Mortensen A, Milstein D, Müller KM. 2011. A new look at an ancient order: generic revision of the Bangiales (Rhodophyta). *Journal of Phycology* **47**: 1131–1151.

Suzuki K, Ohta N, Kuroiwa T. 1992. Isolation of the cell-nuclear, mitochondrial and chloroplast DNA from the ultra-small eukaryote *Cyanidioschyzon merolae*. *Protoplasma* **171**: 80–84.

Tai V, Lindstrom SC, Saunders GW. 2001. Phylogeny of the Dumontiaceae (Gigartinales, Rhodophyta) and associated families based on SSU rDNA and internal transcribed spacer sequence data. *Journal of Phycology* **37**: 184–196.

Thomas DT, Freshwater DW. 2001. Studies of Costa Rican Gelidiales (Rhodophyta): four Caribbean taxa including *Pterosiphonia beachii* sp. nov. *Phycologia* **40**: 340–350.

Tronchin EM, Freshwater DW. 2007. Four Gelidiales (Rhodophyta) new to southern Africa, *Aphanta pachyrrhiza* gen. et sp. nov., *Gelidium profundum* sp. nov., *Pterocladiella caerulescens* and *P. Psammophila* sp. nov. *Phycologia* **46**: 325–348.

Verbruggen H, Maggs CA, Saunders GW, Le Gall L, Yoon HS, De Clerck O. 2010. Data mining approach identifies research priorities and data requirements for resolving the red algal tree of life. *BMC Evolutionary Biology* **10**: 16. doi:10.1186/1471–2148–10–16.

Vis ML, Sheath RG. 1997. Biogeography of *Batrachospermum gelatinosum* (Batrachospermales, Rhodophyta) in North America based on molecular and morphological data. *Journal of Phycology* **33**: 520–526.

Vis ML, Saunders GW, Sheath RG, Dunse K, Entwisle TJ. 1998. Phylogeny of the Batrachospermales (Rhodophyta) inferred from *rbc*L and 18S ribosomal DNA gene sequences. *Journal of Phycology* **34**: 341–350.

von Stosch HA, Theil G. 1979. A new mode of life history in the freshwater red algal genus *Batrachospermum*. *American Journal of Botany* **66**: 105–107.

Waaland JR, Stiller JW, Cheney DP. 2004. Macroalgal candidates for genomics. *Journal of Phycology* **40**: 26–33.

Wendel JF, Cronn RC, Johnston JS, Price HJ. 2002. Feast and famine in plant genomes. *Genetica* **115**: 37–47.

West JA, Zuccarello GC, Scott J, Pickett-Heaps J, Kim GH. 2005. Observations on *Purpureofilum apyrenoidigerum* gen et sp. nov. from Australia and *Bangiopsis subsimplex* from India (Stylonematales, Bangiophyceae, Rhodophyta). *Phycological Research* **53**: 49–66.

Withall RD, Saunders GW. 2006. Combining small and large subunit ribosomal DNA genes to resolve relationships among orders of the Rhodymeniophycidae (Rhodophyta): recognition of the Acrosymphytales ord. nov. and Sebdeniales ord. nov. *European Journal of Phycology* **41**: 379–394.

Woelkerling WJ. 1990. An introduction. In: Cole KM, Sheath RG, eds. *Biology of the red algae*. Cambridge: Cambridge University Press, 1–6.

Woelkerling WJ, Irvine LM, Harvey AS. 1993. Growth forms in non-geniculate coralline red algae (Corallinales, Rhodophyta). *Australian Systematic Botany* **6**: 277–293.

Womersley HBS. 1996. *The marine benthic flora of southern Australia Part IIIB*. Canberra: South Australian Government Printing.

Wynne MJ, Schneider CW. 2010. Addendum to the synoptic review of red algal genera. *Botanica Marina* **53**: 291–299.

Yoon HS, Hackett JD, Bhattacharya D. 2002a. A single origin of the peridinin- and fucoxanthin-containing plastids in

dinoflagellates through tertiary endosymbiosis. *Proceedings of the National Academy of Sciences of the USA* **99**: 11724–11729.

Yoon HS, **Hackett JD**, **Pinto G**, **Bhattacharya D**. **2002*b***. The single, ancient origin of chromist plastids. *Proceedings of the National Academy of Sciences of the USA* **99**: 15507–15512.

Yoon HS, **Hackett JD**, **Ciniglia C**, **Pinto G**, **Bhattacharya D**. **2004**. A molecular timeline for the origin of phytosynthetic eukaryotes. *Molecular Biology and Evolution* **21**: 809–818.

Yoon HS, **Muller KM**, **Sheath RG**, **Ott FD**, **Bhattacharya D**. **2006**. Defining the major lineages of red algae (Rhodophyta). *Journal of Phycology* **42**: 482–492.

Zuccarello GC, **Sandercock B**, **West JA**. **2002**. Diversity within red algal species: variation in world-wide samples of *Spyridia filamentosa* (Ceramiaceae) and *Murrayella periclados* (Rhodomelaceae) using DNA markers and breeding studies. *European Journal of Phycology* **37**: 403–417.

Appendix I. Chromsome number and nuclear DNA content in isolates and species of Rhodophyta. See the 'Notes to Appendix 1' file for explanations of abbreviations and symbols.

Entry num.	Species[a]	2n[b]	Original ref. for 2n	DNA amount 1C (Mbp)[c]	1C (pg)[d]	2C (pg)[d]	4C (pg)[d]	Original ref. for C-value[e]	Standard species[f]	Method[g]
	CYANIDIOPHYTINA									
	CYANIDIOPHYCEAE									
	CYANIDIALES									
1a	*Cyanidioschyzon merolae* DeLuca, Taddei et Varamo	20	33,52	16.5‡	0.02	0.04	0.08	33		PFGE
1b	*Cyanidioschyzon merolae*			11.7‡	0.01	0.02	0.04	32		PFGE
2a	*Cyanidium caldarium* (Tilden) Geitler			12.0‡	0.012	0.024	0.048	50		PFGE
2b	*Cyanidium caldarium*	10 (-14)	36	14.7	0.015*	0.030	0.060	36		Fe
3	*Galdieria maxima* Sentsova	4	36	24.5	0.025*	0.050	0.10	36	Sac	Fe
4	*Galdieria partita* Sentsova	4	36	54.8	0.056*	0.112	0.224	36	Sac	Fe
5a	*Galdieria sulphuraria* (Galdieri) Merola	4	36	12.7	0.013*	0.026	0.062	36	Sac	Fe
5b	*Galdieria sulphuraria*			9.8‡	0.10	0.20	0.40	35		PFGE
	RHODOPHYTINA									
	COMPSOPOGONOPHYCEAE									
	COMPSOPOGONALES									
6	*Compsopogon caeruleus* (Balbis ex C. Agardh) Montagne	c. 14	40	98	0.1	0.2*	0.4	16	Gallus	MI:DAPI
	ERYTHROPELTIDALES									
7	*Erythrotrichia carnea* (Dillwyn) J. Agardh			295	0.3	0.7*	1.4	16	Gallus	MI:DAPI
	PORPHYRIDIOPHYCEAE									
	PORPHYRIDIALES									
8	*Flintiella sanguinaria* F. D. Ott			196	0.2	0.4*	0.8	°	Gallus	MI:DAPI
	Porphyridium purpureum (Bory de Saint-Vincent) K. Drew et Ross (as *Porphyridium cruentum* (S.F. Gray) Nägeli)	4	48	49	0.1	0.1*	0.2	5		DGC
9										
	RHODELLOPHYCEAE									
	RHODELLALES									
10	*Rhodella violacea* (Kornmann) Wehrmeyer			196	0.2	0.4*	0.8	°	Gallus	MI:DAPI
	STYLONEMATOPHYCEAE									
	STYLONEMATALES									
11	*Rhodosorus marinus* Geitler			196	0.2	0.4*	0.8	°	Gallus	MI:DAPI
12	*Stylonema alsidii* (Zanardini) K. Drew			245	0.3	0.5*	1.0	°	Gallus	MI:DAPI
	BANGIOPHYCEAE									
	BANGIALES									
13	*Bangia atropurpurea* (Roth) C. Agardh	6	49	490	0.5	1.0*	2.0	16	Gallus	MI:H
14	*Porphyra purpurea* (Roth) C. Agardh	10	20	270‡	0.3*	0.6	1.2	29	Gallus	FC:EB
15	*Pyropia elongata* (Kylin) Neefus et J. Brodie (as *Porphyra rosengurtii* Coll et Cox)	6	20	490	0.5*	1.0	2.0	25	Ant-Ara	MI:H
16a	*Pyropia leucosticta* (Thur.) Neefus et J. Brodie (NC) (as *Porphyra leucosticta* Thur. [NC])	8	20	480	0.5*	1.0	2.0	25	Ant-Ara	MI:H
16b	*Pyropia leucosticta* (TX) (as *Porphyra leucosticta* [TX])	6	20	490	0.5*	1.1	2.2	25	Ant-Ara	MI:H
17a	*Pyropia spiralis* var. *amplifolia* (E.C.Oliveira Filho et Coll) Freshwater et Kapraun (as *Porphyra spiralis* var. *amplifolia* E.C.Oliveira et Coll)	8	20	588	0.6*	1.2*	2.4	25	AntAra	MI:H
17b	*Pyropia spiralis* var. *amplifolia* (as *Porphyra spiralis* var. *amplifolia*)	6	20	588	0.6*	1.2*	2.4	25	Ant-Ara	MI:H
18	*Pyropia suborbiculata* (Kjellm.) J.E. Sutherland, H.G. Choi, M.S. Hwang et W.A. Nelson (as *Porphyra carolinensis* Coll et Cox)	8	21	490	0.5*	1.0	2.0	21	Ant-Ara	MI:H
	FLORIDEOPHYCEAE									

	NEMALIOPHYCIDAE									
	ACROCHAETIALES									
19	*Audouinella botryocarpa* (Harvey) Woelkerling			588	0.6[(1)]	1.3*	2.6	16	Gallus	MI:DAPI
20	*Audouinella thuretii* (Bornet) Woelkerling			588	0.6*[(1)]	1.2	2.4	16	Gallus	MI:DAPI
21	*Rhodochorton purpureum* (Lightfoot) Rosenvinge			440	0.5	0.9*		°	Gallus	MI:DAPI
	(as *Rhodochorton tenue* Kylin)									
	BATRACHOSPERMALES									
	Batrachospermaceae									
22	*Batrachospermum antipodites* Entwisle	8	20	122	0.2	0.3	0.5*	17	Gallus	MI:DAPI
	(as *Batrachospermum ectocarpum* Entwisle et Kraft)									
23a	*Batrachospermum gelatinosum* (L.) De Candolle	6	47	196	0.2	0.4*	0.8	°	Gallus	MI:DAPI
23b	*Batrachospermum gelatinosum*			196	0.2	0.4*	0.8	°	Gallus	MI:DAPI
	(as *Batrchospermum moniliforme* Roth)			122	0.2	0.3	0.5*	17	Gallus	MI:DAPI
24	*Batrachospermum helminthosum* Bory de Saint-Vincent	8	47	112	0.1	0.2*	0.4	17	Gallus	MI:DAPI
	(as *Batrachospermum sirodotia* Skuja ex Fint)									
25	*Batrachospermum turfosum* Bory de Saint-Vincent	8	38	196	0.2	0.4*	0.8	17	Gallus	MI:DAPI
	(as *Batrachospermum vagum* (Roth) C. Agardh)									
26a	*Batrachospermum vagum* (Roth) C. Agardh			293	0.3	0.6*	1.2	°	Gallus	MI:DAPI
26b	*Batrachosprmum vagum*			245	0.3	0.5	1.0*	°	Gallus	MI:DAPI
27	*Sirodotia suecica* Kylin	6	38	98	0.1	0.2*	.4	17	Gallus	MI:DAPI
	(as *Sirodotia tenuissima* (Collins) Skuja ex L. H. Flint)			98	0.1	0.2	0.4*	17	Gallus	MI:DAPI
28	*Tuomeya americana* (Kützing) Papenfuss	16	53,60	171	0.2	0.4	0.7*	17	Gallus	MI:DAPI
	(as *Tuomeya fluviatilis* (Linnaeus) C. Agardh)			147	0.2	0.3*	0.6	17	Gallus	MI:DAPI
	Lemaneaceae									
29a	*Lemanea sp*. (Ott #3)			758	0.8	1.5	3.1*	17	Gallus	MI:DAPI
29b	*Lemanea sp*. (Ott #3)			763	0.8	1.6*	3.1	17	Gallus	MI:DAPI
29c	*Lemanea sp*. (Ott #4)			782	0.8	1.6	3.2*	17	Gallus	MI:DAPI
29d	*Lemanea sp*. (Ott #5)			782	0.8	1.6	3.2*	17	Gallus	MI:DAPI
30	*Lemanea fluviatilis* (Linnaeus) C. Agardh	c.36	53							
31a	*Lemanea torulosa* (Roth) C. Agardh			569	0.6	1.2*	2.4	°	Gallus	MI:DAPI
31b	*Lemanea torulosa*			538	0.6	1.1	2.2*	°		
32	*Paralemanea annulata* (F. T. Kützing) Vis et Sheath	20	37	783	0.8	1.6	3.2*	17	Gallus	MI:DAPI
	(as *Lemanea australis* Atkinson)									
33a	*Paralemanea catenata* (Kützing) Vis et Sheath	30	39	489	0.5	1.0	2.0*	17	GAllus	MI:DAPI
	(as *Paralemanea pleocarpa* :Ott 1508)									
33b	*Paralemanea catenata*			538	0.6	1.1*	2.2	°	Gallus	MI:DAPI
33c	*Paralemanea catenata*			489	0.5	1.0	2.0*	°	Gallus	MI:DAPI
33d	*Paralemanea catenata* (CZ-10)			538	0.6	1.1*	2.2	°	Gallus	MI:DAPI
33e	*Paralemanea catenata* (CZ-10)			465	0.5	1.0	1.9*	°	Gallus	MI:DAPI
	COLACONEMATALES									
34	*Colaconema daviesii* (Dillwyn) Stegenga			294	0.3[(1)]	0.6*	1.2	16	Gallus	MI:DAPI
	(as *Audouinella daviesii* (Dillwyn) Woelkerling)									
	NEMALIALES									
35	*Cumagloia andersonii* (Farlow) Setchell et Gardner			391	0.4	0.8*	1.6	°	Gallus	MI:DAPI
36	*Dermonema pulvinatum* (Grunow ex Holmes) Fan					0.7*		16	Gallus	MI:DAPI
	(as *Nemalion pulvinatum* Grunow ex Holmes)									
37	*Dichotomaria marginata* (Ellis et Solander) Lamarck			1223	1.3	2.5*	5.0	°	Gallus	MI:DAPI
	(as *Galaxaura marginata* (Ellis et Solander) Lamouroux)									
38	*Dichotomaria obtusata* (Ellis et Solander) Lamarck			636	0.7	1.3*	2.6	°	Gallus	MI:DAPI
	(as *Galaxaura obtusata* (Ellis et Solander) Lamouroux)									
39	*Galaxaura rugosa* (Ellis et Solander) Lamouroux			1084	1.1[(1)]	2.3*	4.6	16	Gallus	MI:DAPI
40	*Nemalion helminthoides* (Velley) Batters			284	0.3	0.6*	1.2	°	Gallus	MI:DAPI
41	*Scinaia complanata* (Collins) Cotton			245	0.3	0.5*	1.0	°	Gallus	MI:DAPI
	PALMARIALES									
42	*Devaleraea ramentacea* (Linnaeus) Guiry			734	0.8	1.5*	3.0	°	Gallus	MI:DAPI
	(as *Halosaccion ramentaceum* (Linnaeus) J. Agardh)									
43	*Palmaria palmata* (Linnaeus) Kuntze	38-48	54	794	0.8[(1)]	1.6*	3.3*	16	Gallus	MI:DAPI

44	*Rhodothamniella floridula* (Dillwyn) J. Feldmann in Christensen			1176	1.4[(1)]	2.8*	5.6	°	Gallus	MI:DAPI
	(as *Audouinella floridula* (Dillwyn) Woelkerling)									
	THOREALES									
45	*Thorea riekei* Bischoff			147	0.2	0.3*	0.6	17	Gallus	MI:DAPI
	CORALLINOPHYCIDAE									
	CORALLINALES									
46	*Amphiroa beauvoisii* Lamouroux			343	0.3	0.7*	1.4	2	Gallus	MI:DAPI
47	*Amphiroa zonata* Yendo			294	0.3	0.6*	1.2	2	Gallus	MI:DAPI
48	*Bossiella orbigniana* ssp. *dichotoma* (Manza) Johansen			588	0.6	1.2*	1.4	2	Gallus	MI:DAPI
49	*Calliarthron tuberculosum* (Postels et Ruprecht) Dawson			637	0.6	1.3*	1.6	2	Gallus	MI:DAPI
50	*Cheilosporum sagittatum* (Lamouroux) Areschoug			343	0.3	0.7*	1.4	2	Gallus	MI:DAPI
51	*Corallina officinalis* Linnaeus	48	49	588	0.6	1.2*	2.4	2	Gallus	MI:DAPI
52	*Corallina vancouveriensis* Yendo			637	0.6	1.3*	2.6	2	Gallus	MI:DAPI
53	*Heydrichia wolkerlingii* Townsend, Chamberlain et Woelkerling			69	0.1	0.1*	0.2	2	Gallus	MI:DAPI
54	*Heydrichia sp*.			98	0.1	0.2*	0.4	2	Gallus	MI:DAPI
55	*Jania adhaerens* Lamouroux			539	0.6	1.1*	2.2	2	Gallus	MI:DAPI
56	*Leptophytum ferox* (Foslie) Chamberlain et Keats			98	0.1	0.2*	0.4	2	Gallus	MI:DAPI
57	*Lithothrix aspergillum* Gray			343	0.3	0.7*	1.4	2	Gallus	MI:DAPI
58	*Mesophyllum discrepans* Foslie			118	0.1	0.2 *	0.4	2	Gallus	MI:DAPI
59	*Metagoniolithon radiatum* (Lamarck) Ducker			343	0.3	0.7*	1.4	2	Gallus	MI:DAPI
60	*Neogoniolithon spectabile* (Foslie) Setchell et Mason			394	0.4[(1)]	0.8	1.5*	2	Gallus	MI:DAPI
61	*Spongites yendoi* (Foslie) Chamberlain			147	0.1	0.3*	0.6	2	Gallus	MI:DAPI
62	*Titanoderma polycephalum* Foslie			196	0.2	0.4 *	0.8	2	Gallus	MI:DAPI
63	*Titanoderma pustulatum* (Lamouroux) Nägeli			490	0.5	1.0*	2.0	2	Gallus	MI:DAPI
	RHODYMENIOPHYCIDAE									
	BONNEMAISONIALES									
64	*Asparagopsis armata* Harvey	20	51	342	0.4	0.7*	1.4	46	Gallus	MI:DAPI
	(as *Falkenbergia rufolanosa* (Harvey) Schmitz)			379	0.4	0.8	1.6*	46	Gallus	MI:DAPI
65	*Asparagopsis taxiformis* (Delile) Trevisan			379	0.4	0.8	1.6*	46	Gallus	MI:DAPI
	(as *Falkenbergia hillebrandii* (Bornet) Falkenberg)			379	0.4	0.8	1.6*	46	Gallus	!A:DAPI
66	*Bonnemaisonia asparagopsis* (Woodward) C. Agardh	36-60	45	293	0.3	0.6*	1.2	46	Gallus	MI:DAPI
	(as *Hymenoclonium serpens* (Crouan frat.) Batters)			293	0.3	0.6	1.2*	46	Gallus	IA:DAPI
	(as *Hymenoclonium serpens* (Crouan frat.) Batters)			269	0.3	0.6	1.1*	46	Gallus	MI:DAPI
67	*Bonnemaisonia clavata* Hamel			293	0.3	0.6*	1.2	46	Gallus	MI:DAPI
	(as *Hymenoclonium serpens* (Crouan frat.) Batters)			293	0.3	0.6	1.2*	46	Gallus	IA:DAPI
68	*Bonnemaisonia hamifera* Hariot	40+	31	379	0.4	0.8*	1.6	46	Gallus	MI:DAPI
	(as *Trailliella intricata* Batters)			318	0.4	0.7	1.3*	46	Gallus	MI:DAPI
69	*Delisea plumosa* Levring			245	0.3	0.5	1.0*	°	Gallus	MI:DAPI
70	*Ptilonia willana* Lindauer			293	0.3	0.6*	1.2	°	Gallus	MI:DAPI
	CERAMIALES									
	Callithamniaceae									
71	*Aglaothamnion boergesenia* (Aponte et Ballantine) L'Ardy-Halos et Rueness	60	11	1372	1.4	2.8*	5.6	18	Gallus	MI:DAPI
	(as *Callithamnion byssoides* Arnott ex Harvey)									
72	*Aglaothamnion callophyllidicola* Boo, I.K.Lee, Rueness et Yoshida			1130	1.2	2.31*	4.6	6		NV
	(as *Callithamnion callophyllidicola* Yamada)									
73	*Aglaothamnion cordatum* (Børgesen)Geldmann-Mazoyer			830	0.9	1.8	3.5*	°	Gallus	MI:DAPI
	(as *Callithamnion cordatum* Børgesen)									
74	*Aglaothamnion halliae* (F.S.Collins) Aponte, Ballantine et Norris			611	0.7	1.3	2.5*	°	Gallus	MI:DAPI
75	*Crouania attenuata* (C. Agardh) J. Agardh			392	0.4[(1)]	0.9*	1.8	16	Gallus	MI:DAPI
76	*Crouania pleonospora* W. Taylor			931	0.9[(1)]	1.8	3.3*	16	Gallus	MI:DAPI
	Ceramiaceae *sensu stricto*									
77	*Antithamnion aglandum* Kim et I.K. Lee			660	0.7	1.35*	2.7	6		NV
78	*Antithamnion callocladum* Itono			694	0.7	1.42*	2.8	6		NV
79	*Antithamnion densum* Kylin			420	0.4	0.86*	1.7	6		NV
80	*Antithamnion pectinatum* (Montagne) Brauner			660	0.7	1.3*	2.6	6		NV
81	*Antithamnion sparsum* Tokida			949	1.0	1.94*	3.9	6		NV
82	*Antithamnion villosum* (Kützing) Athanasiadis in Maggs et Hommers.	48	13	980	1.0	2.0*	4.0	18	Gallus	MI:DAPI

	(as *Antithamnion cruciatum* (C. Agardh) Nägeli)									
83	*Centroceras clavulatum* (C. Agardh) Montagne			588	0.6[(1)]	1.2*	2.4	16	Gallus	MI:DAPI
84	*Ceramium cimbricum* H. Petersen			392	0.4[(1)]	0.8	1.6*	16	Gallus	MI:DAPI
85	*Ceramium strictum* Harvey			245	0.3	0.5*	1.0	16	Gallus	MI:DAPI
86	*Pterothamnion yezoense* (Inagaki) Athanasiadis			220	0.2	0.45*	0.9	6		NV
	Dasyaceae									
87	*Dasya baillouviana* (S. G. Gmelin) Montagne	40	44	490	0.5	1.0*	2.0	18	Gallus	MI:DAPI
88	*Dasya collabens* Hooker et Harvey			220	0.2	0.45*	0.9	6		NV
89	*Dasya ocellata* (Grateloup) Harvey in Hooker			931	0.9[(1)]	1.8	3.5*	16	Gallus	MI:DAPI
90	*Dasya rigidula* (Kützing) Ardissone			709	0.7	1.4	2.9*	°	Gallus	MI:DAPI
91	*Dasya villosa* Harvey					0.57*		6		NV
92	*Heterosiphonia crispella* (C. Agardh) Wynne			318	0.4	0.7	1.3*	°	Gallus	MI:DAPI
93	*Heterosiphonia gibbesii* (Harvey) Falkenberg			394	0.4[(1)]	0.8*	1.6	16	Gallus	MI:DAPI
94	*Heterosiphonia japonica* Yendo	ca.60	41	196	0.2	0.40*	0.8	6		NV
95	*Heterosiphonia pulchra* (Okamura) Falkenberg			249	0.3	0.51*	1.0	6		NV
	Delesseriaceae									
96	*Caloglossa leprieurii* (Montagne) J. Agardh			590	0.6	1.2*	2.4	16	Gallus	MI:DAPI
97	*Calonitophyllum medium* (Hoyt) Aregood			690	0.7[(1)]	1.4	2.9*	16	Gallus	MI:DAPI
98	*Grinnellia americana* (C. Agardh) Harvey			588	0.6[(1)]	1.2	2.4*	16	Gallus	MI:DAPI
99	*Hypoglossum tenuifolium* (Harvey) J. Agardh			586	0.7[(1)]	1.4	2.9*	16	Gallus	MI:DAPI
100	*Martensia fragilis* Harvey			394	0.4[(1)]	0.9*	1.8	16	Gallus	MI:DAPI
	Rhodomelaceae									
101	*Acanthophora spicifera* (Vahl) Børgesen	64	8	490	0.5	1.1*	2.1	16	Gallus	MI:DAPI
102	*Bostrychia moritziana* (Sonders) J. Agardh			690	0.7[(1)]	1.3	2.7*	16	Gallus	MI:DAPI
103	*Bostrychia radicans* (Montagne) Montagne			931	0.9[(1)]	1.8	3.5*	16	Gallus	MI:DAPI
104	*Bryothamnion seaforthii* (Turner) Kützing			490	0.5[(1)]	1.0*	2.0	16	Gallus	MI:DAPI
105	*Chondria dasyphylla* (Woodward) C. Agardh	62	42	490	0.5	1.0*	2.0	18	Gallus	MI:DAPI
106	*Chondria littoralis* Harvey			490	0.5	1.1*	2.2	18	Gallus	MI:DAPI
107	*Murrayella periclados* (C. Agardh) Schmitz			586	0.7[(1)]	1.4	2.8*	16	Gallus	MI:DAPI
108	*Neosiphonia harveyi* (Bailey) M.-S. Kim, H.-G. Choi, Guiry et G.W. Saunders	64	12	1029	1.1	2.1*	4.2	15	Gallus	MI:DAPI
	(as *Polysiphonia harveyi* Bailey)									
109	*Neosiphonia sphaerocarpa* (Børgesen) M.S.Kim et I.K.Lee			539	1.1	2.2*	4.4	15	Gallus	MI:DAPI
	(as *Polysiphonia sphaerocarpa* Børgesen)									
110	*Palisada perforata* (Bory de Saint-Vincent) K.W. Nam	40	56	833	0.8[(1)]	1.6	3.1*	16	Gallus	MI:DAPI
	(as *Laurencia papillosa* (C. Agardh) Greville)									
111	*Polysiphonia boldii* Wynne et Edwards			833	0.8	1.7*	3.4	15	Gallus	MI:DAPI
112	*Polysiphonia elongata* (Hudson) Sprengel	74	1	637	0.7	1.3*	2.6	15	Gallus	MI:DAPI
113	*Polysiphonia fibrillosa* (Dillwyn) Sprengel	32	43	833	0.8	1.7*	3.4	15	Gallus	MI:DAPI
	(as *Polysiphonia violacea* (Roth) Sprengel									
114	*Polysiphonia fucoides* (Hudson) Greville	60	1	539	0.6	1.1*	2.2	15	Gallus	MI:DAPI
	(as *Polysiphonia nigrescens* (Hudson) Greville)									
115	*Polysiphonia kapraunii* Stuercke et Freshwater	60	15	392	0.44	0.8*	1.6	15	Gallus	MI:DAPI
	(as *Polysiphonia urceolata* Lightfoot ex Dillwyn sensu Kapraun 1977)									
116	*Polysiphonia opaca* (C. Agardh) Moris et De Notaris	60	1	784	0.8	1.6*	3.2	15	Gallus	MI:DAPI
117	*Polysiphonia schneideri* Stuercke et Freshwater	60	15	931	0.9	1.9*	3.8	15	Gallus	MI:DAPI
	(as *Polysiphonia denudata* (Dillwyn) Greville ex Harvey in Hooker)									
118	*Polysiphonia stricta* (Dillwyn) Greville sensu Taylor 1957			784	0.8	1.6*	3.2	15	Gallus	MI:DAPI
	(as *Polysiphonia urceolata* Lightfoot ex Dillwyn)									
119	*Wrightiella tumanowiczii* (Gaty ex Harvey) F. Schmitz			685	0.7	1.4*	2.8	°	Gallus	MI:DAPI
	Spyridiaceae									
120	*Spyridia filamentosa* (Wulfen) Harvey in Hooker			833	0.8[(1)]	1.5*	3.0	16	Gallus	MI:DAPI
	Wrangeliaceae									
121	*Anotrichium multiramosum* (Setchell and Gardner) Baldock			394	0.4[(1)]	0.8	1.5*	16	Gallus	MI:DAPI
	Griffithsia japonica Okamura			127	0.1	0.26*	0.5	6		NV
122	*Wrangelia penicillata* (C. Agardh) C. Agardh			931	0.9[(1)]	1.8	3.6*	16	Gallus	MI:DAPI
	GELIDIALES									
123	*Gelidiella acerosa* (Forsskål) Feldmann et Hamel	12	27	147	0.2	0.3	0.6*	27	Gallus	MI:DAPI
124	*Gelidium americanum* (Taylor) Santelices	24	23	294	0.3	0.6*	1.1	9	Gallus	MI:DAPI
125	*Gelidium coulteri* Harvey			245	0.3	0.5	0.9*	9	Gallus	MI:DAPI
126	*Gelidium crinale* (Turner) Lamouroux			294	0.3	0.65*	1.2	9	Gallus	MI:DAPI

	(as *Gelidium pusillum* (Stackhouse) Le Jolis)									
127	*Gelidium floridanum* W. R. Taylor	12	23	294	0.3	0.6	1.1*	9	Gallus	MI:DAPI
128	*Gelidium robustum* (Gardner) Hollenberg et Abbott			294	0.3	0.6*	1.2	9	Gallus	MI:DAPI
129	*Gelidium serrulatum* J. Agardh	20	23	196	0.2	0.4*	0.8	9	Gallus	MI:DAPI
130	*Pterocladiella capillacea* (S. G. Gmellin) Santecles et Hommersand (as *Pterocladia capillacea* (S. G. Gmellin) Bornet et Thuret)	20	23	245	0.3	0.5*	1.0	24	Gallus	MI:DAPI
131	*Pterocladiella melanoidea* (Schousboe et Bornet) Santelices et Hommersand			343	0.3	0.7*	1.4	9	Gallus	MI:DAPI
	GIGARTINALES									
132	*Agardhiella subulata* (C. Agardh) Kraft et Wynne	44	24	441	0.4	0.9	1.9*	26	Gallus	MI:DAPI
133	*Ahnfeltiopsis concinna* (J. Agardh) P. C. Silva et DeCew			147	0.2[(1)]	0.3*	0.6	18	Gallus	MI:DAPI
134a	*Chondrus crispus* Stackhouse	64-70	10	98	0.1	0.2	0.5*	18	Gallus	MI:DAPI
134b	*C. crispus*			98	0.1	0.2*	0.4	32	Gallus	FC:EB
134c	*C. crispus*			105‡				7		WGS
135	*Dudresnaya crassa* Howe			245	0.3	0.5*	1.0	°	Gallus	MI:DAPI
136	*Dudresnaya georgiana* Searles			245	0.3	0.5*		°	Gallus	MI:DAPI
137	*Dumontia contorta* (S. G. Gmelin) Ruprecht	22-24	31	196	0.2[(1)]	0.4*	0.8	16	Gallus	MI:DAPI
138	*Eucheuma denticulatum* (N. L. Burman) Collins et Hervey	20	21	147	0.1	0.3*	0.6	21	Gallus	MI:DAPI
139	*Eucheuma isiforme* (C. Agardh) J. Agardh			196	0.2	0.4*	0.8	21	Gallus	MI:DAPI
140	*Gymnogongrus griffithsiae* (Turner) Martius	46	26	147	0.1	0.3*	0.6	26	Gallus	MI:DAPI
141	*Hypnea musciformis* (Wulfen in Jacquin) Lamouroux	10	22	147	0.1	0.2*	0.4	22	Gallus	MI:DAPI
142a	*Kappaphycus alvarezii* (Doty) Doty	20	21	147	0.1	0.3	0.5*	21	Gallus	MI:DAPI
142b	*K. alvarezii*			196‡	0.2	0.4*	0.8	29	Gallus	FC:EB
143	*Kappaphycus striatum* (Schmitz) Doty			196	0.2	0.4*	0.8	21	Gallus	MI:DAPI
144	*Soliera filiformis* (Kützing) Gabrielson			197	0.2[(1)]	0.4*	0.8	16	Gallus	MI:DAPI
	GRACILARIALES									
145	*Gracilaria arcuata* Zanardini			186	0.2	0.4*	0.8	28	Gallus	MI:DAPI
146	*Gracilaria blodgettii* Harvey			186	0.2	0.4*	0.8	30	Gallus	MI:DAPI
147	*Gracilaria caudata* J. Agardh			196	0.2	0.4*	0.8	30	Gallus	MI:DAPI
148	*Gracilaria cervicornis* (Turner) J. Agardh			196	0.2	0.4*	0.8	30	Gallus	MI:DAPI
149	*Gracilaria divaricata* Harvey			196	0.2	0.4*	0.8	30	Gallus	MI:DAPI
150	*Gracilaria eucheumoides* Harvey			206	0.2	0.4*	0.8	28	Gallus	MI:DAPI
151	*Gracilaria firma* Zhang et Xia			196	0.2	0.4*	0.8	28	Gallus	MI:DAPI
152	*Gracilaria flabelliforme* P. Crouan et H. Crouan ex Schramm et Maze	48	14	191	0.2	0.4*	0.8	14	Gallus	MI:DAPI
153	*Gracilaria mammillaris* (Montagne) M. A. Howe	48	14	196	0.2	0.4*	0.8	14	Gallus	MI:DAPI
154	*Gracilaria pacifica* Abbott	48	4,14	196	0.2	0.4*	0.8	14	Gallus	MI:DAPI
155	*Gracilaria salicornia* (C. Agardh) E. Y. Dawson			191	0.2	0.4*	0.8	28	Gallus	MI:DAPI
156	*Gracilaria tikvahiae* McLachlan	48	34	191	0.2	0.4*	0.8	14	Gallus	MI:DAPI
157	*Gracilaria sp*. (NC)			196	0.2	0.4	0.8*	16	Gallus	MI:DAPI
158	*Gracilariopsis bailinae* Zhang et Xia			196	0.2	0.4*	0.8	28	Gallus	MI:DAPI
159	*Gracilariopsis carolinensis* Liao et Hommersand (as *Gracilariopsis lemaneiformis* (Bory) Dawson, Acleto et Foldvik)	64	14	196	0.2	0.4*	0.8	14	Gallus	MI:DAPI
160	*Gracilariopsis longissima* (S.G. Gmelin) M. Steentoft, L.M. Irvine & W.F. Farnham (as *Gracilaria verrucosa* (Hudson) Papenfuss)	48	3	147	0.2	0.3*	0.6	14	Gallus	MI:DAPI
161	*Gracilariopsis tenuifrons* (Bird et Oliveira) Fredericq et Hommersand	64	14	196	0.2	0.4*	0.8	14	Gallus	MI:DAPI
162	*Hydropuntia cornea* (J. Agardh) Wynne			245	0.2	0.5*	1.0	14	Gallus	MI:DAPI
163	*Hydropuntia dentata* (J. Agardh) Wynne			196	0.2	0.4*	0.8	14	Gallus	MI:DAPI
164	*Hydropuntia fastigiata* (Zhang et Xia) Wynne			196	0.2	0.4*	0.8	28	Gallus	MI:DAPI
	HALYMENIALES									
165	*Grateloupia filicina* (Lamouroux) C. Agardh			196	0.2*[(1)]	0.4	0.8	16	Gallus	MI:DAPI
166	*Halymenia floridana* J. Agardh			196	0.2*[(1)]	0.4	0.8	16	Gallus	MI:DAPI
	NEMASTOMATALES									
167	*Predaea feldmannii* Børgesen			147	0.2	0.3*	0.6	°	Gallus	MI:DAPI
168	*Predaea masonii* (Setchell et Gardner) G. de Toni			147	0.2	0.3*	0.6	°	Gallus	MI:DAPI
	RHODYMENIALES									
169	*Champia affinis* (J.D. Hooker et Harvey) Harvey			237	0.3	0.5	1.0*	°	Gallus	MI:DAPI
170	*Champia chathamensis* V. J. Chapman et Dromgoole			293	0.3	0.6*	1.2	°	Gallus	MI:DAPI

171	*Champia parvula* (C. Agardh) Harvey	24	18	196	0.2	0.4*	0.8	18	Gallus	MI:DAPI
172	*Chrysemenia enteromorpha* Harvey			391	0.2	0.4	0.8*	°	Gallus	MI:DAPI
173	*Gloioderma saccatum* (J. Agardh) Kylin			237	0.3	0.5	0.9*	°		
174	*Hymenocladia sanguinea* (Harvey) Sparling			293	0.2	0.4*	0.8	°		
175	*Lomentaria baileyana* (Harvey) Farlow	20	18	196	0.2	0.3*	0.6	18	Gallus	MI:DAPI
176	*Rhodymenia divaricata* Dawson			293	0.3	0.6*	1.2	°	Gallus	MI:DAPI
177	*Rhodymenia pseudopalmata* (Lamouroux) Silva	20	18	294	0.3	0.5*	1.0	18	Gallus	MI:DAPI
	SEBDENIALES									
178	*Sebdenia flabellata* (J. Agardh) Parkinson			245	0.3	0.5*	1.0	°	Gallus	MI:DAPI

File 2. Notes on Appendix I.

(a) Taxa are listed alphabetically within orders. Assignment of species to specific orders and families follows http://algaebase.org. Continuing molecular systematic investigations impact on our understanding of the delineation and composition of taxa at all levels: orders, family and genera. An attempt has been made to assign genera to currently recognized families, but on-going molecular investigations have demonstrated that many of these families are not natural assemblages. Synonyms are provided in cases where chromosome complements and/or nuclear DNA content estimates were originally published under different genus and/or species epithets. Footnotes are provided in Appendix 1 for some of these examples and footnote references are included at the end of this file.

(b) Most comprehensive lists of chromosome numbers have been published as haploid (1*n*) values for the Rhodophyta (Cole 1990). In the Appendix, chromosome numbers are extrapolated from 1*n* numbers (and ranges of probable 1*n* numbers).

(c) Since most DNA amounts in the literature are given in picograms (pg), unless otherwise indicated, Mbp values in Appendix 1 are derived, using the expression 1 pg = 978 Mbp (Dolezel *et al*. 2003). DNA amounts for taxa originally published as megabase pairs (Mbp) are indicated with a '‡'.

(d) Algal life histories typically are characterized by an alternation of haploid gametophyte and diploid sporophyte generations (Kapraun 1993, Kapraun and Dunwoody 2002). Thus, DNA content (pg) measurements could be based on either or both 2C replicated haploid nuclei or 4C replicated diploid nuclei. In some samples, available specimens were not reproductive and ploidy level could not be determined with certainty. Assignment of DNA content to specific C-level for these isolates is speculative [(1)]. In practice, most published DNA content (pg) values are for 2C diploid nuclei and most 1C and 4C values are extrapolated.

In Appendix 1, the original published DNA content (pg) value for each species is indicated with an asterisk (*). Additional DNA contents, both pg and Mbp, are derived. Estimates for replicated (2C) haploid (1n) genomes are given to only one decimal space and should be considered accurate only to ± 0.1 pg (Kapraun 2005, 2007). DNA values for some Cyanidiales (Mbp †) are expressed to three decimal places as published (Muravenko *et al*. 2001). DNA values for some Ceramiales are expressed to two decimal places (Choi *et al*. 2004) as published.

As all values are calculated from the single orginial value (pg* or Mbp‡), some derived values in Appendix 1 appear to reflect a lack of precision. For example, an original value of 4C = 0.5 pg for *Batrachospermum antipodes* becomes 2C = 0.3 pg (not 0.25 pg) and 1C = 0.2 pg (not 0.13 pg) or 122 Mbp.

(e) Previously unpublished data are indicated as an open circle (○). Information for collection locations, and data for number of algal nuclei examined in each sample and estimates of nuclear genome size (pg) ± SD are available at http://www.uncw.edu/people/kapraund/ DNA.

(f) Standard species

The vast majority of nuclear DNA estimates for algae have used chicken (*Gallus gallus*) red blood cells or erythrocytes (RBC) for a DNA standard (listed as Gallus in column 10 of Appendix I) despite limitations of RBC as a standard for plant material as discussed elsewhere (Johnston *et al*. 1999, Bennett *et al*. 2000). When our program of nuclear DNA quantification of algae with microspectrophotometry was initiated in 1990 (Kapraun and Shipley 1990), the 2C DNA content of *Gallus gallus* was reported to be within the range of 2.33 (Galbraith *et al*. 1983) to 2.39 pg (Clowes *et al*. 1983), with 2.4 pg being a generally accepted value (Kapraun 2005). More accurate quantification techniques now estimate the 2C DNA content of RBC to be 2.5 pg (Bennett *et al.* 2003; Gregory 2005). However, an editorial decision was made to continue use of the RBC = 2.4 pg in all subsequent algal publications

(*e.g*. Kapraun 2005, 2007) to avoid confusion with previous data incorporated into the RBG plant genome sizes data base (Bennett and Leitch 2005). It should be noted that as all algal DNA content data are given only to the nearest ± 0.1 pg, use of 2.4 instead of 2.5 pg as RBC standard (a difference of less than a 4%) rarely alters reported values.

Initial investigations in our laboratory (summarized in Kapraun 2005) utilized a standard line based on the fluorescence intensity of the angiosperms *Antirrhinum majus* L. (Ant) and *Arabidopsis thaliana* (Linnaeus) Heynhold (Ara). Recently, Choi *et al*. (2004) used the angiosperms *Capsicum annuum* Linnaeus and *Nicotiana tabacum* Linnaeus as standards in their investigation of ceramialean algae. *Saccharomyces cerevisiae* was used to standardize Feulgen arbitrary units for the very small small genomes in some cyanidiophytes (Muravenko *et al*. 2001).

(g) Methods:

One of the earliest estimates of cellular DNA content in red algae was made for *Porphyridium* using cesium chloride density gradient centrifugation (DGC) (Charles 1977). Contemporary techniques include static cytometry or microspectrophotometry (MI:DAPI) (Kapraun 1994, Kapraun and Buratti 1998) and image analysis (IA:DAPI) (Salvador Soler *et al.* 2011) which have been shown to be reliable methods for quantification of nuclear DNA contents in the Rhodophyta (Kapraun 2005; Kapraun *et al*. 2007). Recently, Choi *et al*. (2004) estimated nuclear DNA contents using nuclear volume (NV) measurements derived from DAPI-stained spermatial nuclei as an alternative to more expensive flow cytometry techniques. Specifically, DNA contents were determined using the expression DNA = NV/15μm^3 x 1 pg (Kapraun and Nguyen 1994, Choi and Lee 1996, Choi and Kim 2001). The nuclear genome project for *Chondrus crispus* (Collén J. 2011) used whole genome sequencing (WGS) techniques as described in detail for *Ectocarpus siliculosus* (Dillwyn) Lyngbye (Cock *et al.* 2010).

The particularly small nuclear genomes in the Cyanidiales have been quantified using pulse-field gel electrophoresis (PFGE) (Matsuzaki *et al*. 2004). In this technique, the small chromosomes are separated in an agarose gel and their sizes quantified and added. However, it can be difficult to

distinguish chromosomes of similar size (Matsuzaki *et al*. 2004). Feulgen microspectrophotometry (Fe) has been used with Cyanidiales as well, with Feulgen arbitrary units converted to picograms of DNA by comparison with *Saccharomyces cerevisiae* with a DNA content of 0.1×10^{-2} pg (Sherman 1991; Muravenko *et al*. 2001).

Several DNA-localizing fluorochromes have been used in published investigations. DAPI (4', 6-diamidino-2-phenylindole) is certainly the most popular, especially in recent studies (Kapraun 1994, Kapraun and Buratti 1998). Hydroethidine (H) (Kapraun and Bailey 1992) was used in several preliminary investigations (Kapraun 2005).

Recently, the Angiosperm Genome Size Workshop (Bennett *et al*. 2000) identified 'best practice' methodology for nuclear genome size estimation in plant tissues (For details and recommendations, see http://www.rbgkew.org.uk/cval/conference.html under Angiosperm Genome Size Discussion Meeting). Virtually none of the published genome size data for algae resulted from investigations adhering to all of the best practice recommendations. Even in cases where the preferred methodology of Feulgen microdensitometry was employed, researchers typically used animal (RBC) rather than plant (*Allium* or *Pisum*) standards. Consequently, all present and previously published data included in the Appendix should be considered accurate only to ± 0.1 pg (Kapraun 2005).

References

Bennett MD, Leitch IJ. 2005. Plant genome size research: a field in focus. *Annals of Botany* **95:** 1-6.

Bennett MD, Bhandol P, Leitch IJ. 2000. Nuclear DNA amounts in angiosperms and their modern uses - 807 new estimates. *Annals of Botany* **86:** 859-909.

Bennett MD, Leitch IJ, Price HJ, Johnston JS. 2003. Comparisons with Caenorhabditis (~100 Mb) and Drosophila (~175 Mb) using flow cytometry show genome size in Arabidopsis to be ~157 Mb and thus ~25% larger than the Arabidopsis Genome Initiative estimate of ~125 Mb is not ~125 Mb but approaches Drosophila (~175 Mb). *Annals of Botany* **91:** 547-557.

Charles D. 1977. Isolation and characterization of DNA from unicellular algae. *Plant Science Letters* **8:** 35-44.

Choi H-G, Kim KY. 2001. Mixed-phase reproduction of *Dasysiphonia chejuensis* from Korea: nuclear DNA contents and environmental factors. *Algae* **16:** 437-443.

Choi H-G, Lee IK. 1996. Mixed-phase reproduction of *Dasysiphonia chejuensis* (Rhodophyta) from Korea. *Phycologia* **35:** 9-18.

Choi H-G, Lee E-Y, Oh YS, Kim H-S, Lee IK. 2004. Nuclear DNA quantification of some Ceramialean algal spermatia by fluorescence microscopic image processing and their nuclear SSU rDNA sequences. *Algae* **19:** 79-90.

Clowes AW, Reidy MA, Clowes MM. 1983. Kinetics of cellular proliferation after arterial injury. I. Smooth muscle growth in absence of endothelium. *Laboratory Investigations* **49:** 327-333.

Cock JM, Sterck L, Rouzé P, Scornet D, Allen AE, Amoutzias G, Anthouard V, Artiguenave F, Aury J-M, Badger JH, Beszteri B, Billiau K, Bonnet E, Bothwell JH, Bowler C, Boyen C, Brownlee C, Carrano CJ, Charrier B, Cho GY, Coelho SM, Collén J, Corre E, Da Silva C, Delage L, *et al.* 2010. The *Ectocarpus* genome and the independent evolution of multicellularity in brown algae. *Nature* **485:** 617-621.

Cole K. 1990. Chromosomes. In: Cole KM, Sheath RG, eds. *Biology of the Red Algae*. Cambridge: Cambridge University Press, 73-101.

Collén J. 2011. The *Chondrus crispus* genome. In: 4th Congress of the International Society for Applied Phycology, 19-21 June 2011, Halifax, Canada Meeting. Keynote Address, Abstract #4. ISAP2011-halifax.org

Doležel J, Bartos J, Voglmayr H, Greilhuber J. 2003. Nuclear DNA content and genome size of trout and human. *Cytometry* **51:** 127-128.

Galbraith DW, Harkins KR, Maddox JM, Ayers NM, Sharma DP, Firoozabady E. 1983. Rapid flow cytometric analysis of the cell cycle in intact plant tissues. *Science* **220:** 1049-1051.

Gregory TR. 2005. The C-value enigma in plants and animals: a review of parallels and an appeal for partnership. *Annals of Botany* **95:** 133-146.

Johnston JS, Bennett MD, Rayburn AL, Galbraith DW, Price HJ. 1999. Reference standards for determination of DNA content of plant nuclei. *American Journal of Botany* **86:** 609-613.

Kapraun DF. 1993. Karyology and cytophotometric estimation of nuclear DNA variation in several species of *Polysiphonia* (Rhodophyta, Ceramiales). *Botanica Marina* **36:** 507¬516.

Kapraun DF. 1994. Cytophotometric estimation of nuclear DNA contents in thirteen species of the Caulerpales (Chlorophyta). *Cryptogamic Botany* **4:** 410-418.

Kapraun DF. 2005. Nuclear DNA content estimates in multicellular green, red and brown algae: phylogenetic considerations. *Annals of Botany* **95:** 7-44.

Kaparaun DF. 2007. Nuclear DNA content estimates in green algal lineages: Chlorophyta and Streptophyta. *Annals of Botany* **99:** 677-701.

Kapraun DF, Bailey JC. 1992. Karyology and cytophotometric estimation of nuclear DNA variation in seven species of Ulvales (Chlorophyta). *Japanese Journal of Phycology* **40:** 15-26.

Kapraun DF, Buratti JR. 1998. Evolution of genome size in the Dasycladales (Chlorophyta) as determined by DAPI cytophotometry. *Phycologia* **37:** 176-183.

Kapraun DF, Dunwoody JT. 2002. Relationship of nuclear genome size to some reproductive cell parameters in the Florideophycidae (Rhodophyta). *Phycologia* **41:** 507-516.

Kapraun DF, Nguyen MN. 1994. Karyology, nuclear DNA quantification and nucleus-cytoplasmic domain variations in some multinucleate green algae. *Phycologia* **33:** 42 52.

Kapraun DF, Shipley MJ. 1990. Karyology and nuclear DNA quantification in *Bryopsis* (Codiales, Chlorophyta) from North Carolina, USA. *Phycologia* **29:** 443-453.

Kapraun DF, Braly KS, Freshwater DW. 2007. Nuclear DNA content variation in the freshwater red algal orders Batrachospermales and Thoreales (Florideophyceae, Nemaliophycidae). *Phycologia* **46:** 54-62.

Matsuzaki M, Misumi O, Shin-I T, Maruyama S, Takahara M, Miyagishima SY, Mori T, Nishida K, Yagisawa F, Nishida K, Yoshida Y, Nishimura Y, Nakao S, Kobayashi T, Momoyama Y, Higashiyama T, Minoda A, Sano M, Nomoto H, Oishi K, Hayashi H, Ohta F, Nishizaka S, Haga S, Miura S, Morishita T, Kabeya Y, Terasawa K, Suzuki Y, Ishii Y, Asakawa S, Takano H, Ohta N, Kuroiwa H, Tanaka K, Shimizu N, Sugano S, Sato N, Nozaki H, Ogasawara N, Kohara Y, Kuroiwa T. 2004. Genome sequence of the ultrasmall unicellular red alga *Cyanidioschyzon merolae* 10D. *Nature* **428:** 653-657.

Muravenko OV, Selyakh IO, Kononenko NV, Stadnichuk IN. 2001. Chromosome numbers and nuclear DNA contents in the red microalgae *Cyanidium caldarium* and three *Galdieria* species. *European Journal of Phycology* **36:** 227-232.

Salvador Soler N, Gómez Garreta A, Ribera Siguan MA, Kaparaun DF. 2011. Nuclear DNA content variation in life history phases of Bonnemaisoniales (Rhodophyta) from the Spanish coast. (unpubl. res.).

Sherman F. 1991. Getting started with yeast. In: Guthrie C, Fink GR, eds. *Methods in Enzymology* **194**. San Diego: Academic Press, 3-20.

File 3. Numbered references for chromosome complements and DNA content estimates in the Rhodophyta cited in Appendix I.

1. **Austin AP 1956.** Chromosome counts in the Rhodophyceae. Nature (London) 178: 370-371.
2. **Bailey JC, Kapraun DF.** UNC-W, Wilmington, NC, USA, unpubl. res.
3. **Bird CJ, Rice EL. 1990**. Recent approaches to the taxonomy of the Gracilariaceae (Gracilariales, Rhodophyta) and the *Gracilaria verrucosa* problem. Hydrobiologia 204/205: 111-118.
4. **Bird CJ, van der Meer JP, McLachlan J. 1982.** A comment on *Gracilaria verrucosa* (Huds.) Papenf. (Rhodophyta: Gigartinales). Journal of the Marine Biological Association of the United Kingdom 62: 453-459.
5. **Charles D. 1977**. Isolation and characterization of DNA from unicellular algae. Plant Science Letters 8: 35-44.
6. **Choi H-C, Lee E-Y, Oh YS, Kim H-S, Lee IK. 2004**. Nuclear DNA quantification of some Ceramialean algal spermatia by fluorescence microscopic image processing and their nuclear SSU rDNA sequences. Algae 19: 79-90.
7. **Collen J. 2011.** The *Chondrus crispus genome.* In: *4th Congress of the International Society for Applied Phycology, 19-21 June 2011, Halifax Canada Meeting. Keynote Address, Abstract #4,* ISAP2011-halifax.org
8. **Cordeiro-Marino M, Yamaguishi-Tomita N, Yabu H. 1974**. Nuclear divisions in the tetrasporangia of *Acanthophora spicifera* (Vahl) Boergesen and *Laurencia papillosa* (Forsk.) Greville. Bulletin of the faculty of Fisheries, Hokkaido University 25: 79-81.
9. **Freshwater DW. 1993.** Cytophotometric estimation of inter- and intraspecific variation in nuclear DNA content in ten taxa of the Gelidiales (Rhodophyta). Experimental Marine Biology and Ecology 166: 231-239.
10. **Hanic LA. 1973**. Cytology and genetics of *Chondrus crispus* Stackhouse. Proceedings of the Nova Scotia Institute of Science 27 (Suppl.): 23-52.
11. **Harris RE. 1962**. Contribution to the taxonomy of *Callithamnion* Lyngbye emend. Naegeli. Botanica Notiser 115: 18-28.

12. Kapraun DF. 1978. A cytological study of varietal forms in *Polysiphonia harveyi* and *P. ferulacea* (Rhodophyta, Ceramiales). Phycologia 17: 152-156.

13. Kapraun DF. 1989. Karyological investigations of chromosome variation patterns associated with speciation in some Rhodophyta. In: George RY, Hulber AW. eds. Carolina Oceanography Symposium. Washington: National Undersea Research Program Research Report 892, 65-76.

14. Kapraun DF. 1993a. Karyology and cytophotometric estimation of nuclear DNA content variation in *Gracilaria*, *Gracilariopsis* and *Hydropuntia* (Gracilariales, Rhodophyta). European Journal of Phycology 28: 253-260.

15. Kapraun DF. 1993b. Karyology and cytophotometric estimation of nuclear DNA variation in several species of *Polysiphonia* (Rhodophyta, Ceramiales). Botanica Marina 36: 507-516.

16. Kapraun DF. 2005. Nuclear DNA content estimates in multicellular green, red and brown algae: phylogenetic considerations. Annals of Botany 95: 7-44.

17. Kapraun DF, Braly KS, Freshwater DW. 2007. Nuclear DNA content variation in the freshwater red algal orders Batrachospermales and Thoreales (Florideophyceae, Nemaliophyceae). Phycologia 46: 54-62.

18. Kapraun DF, Dunwoody JT. 2002. Relationship of nuclear genome size to some reproductive cell parameters in the Florideophycidae (Rhodophyta). Phycologia 41: 507-516.

19. Kapraun DF, Dutcher JA. 1991. Cytophotometric estimation of inter- and intraspecific nuclear DNA content variation in *Gracilaria* and *Gracilariopsis* (Gracilariales, Rhodophyta). Botanica Marina 34: 139-144.

20. Kapraun DF, Freshwater DW. 1987. Karyological studies of five species in the genus *Porphyra* (Bangiales, Rhodophyta) from the North Atlantic and Mediterranean. Phycologia 26: 82-87.

21. Kapraun DF, Hinson TK, Lemus AJ. 1991. Karyology and cytophotometric estimation of inter- and intraspecific nuclear DNA variation in four species of *Porphyra* (Rhodophyta). Phycologia 30: 458-466.

22. Kapraun DF, Lopez-Bautista J. 1997. Karyology, nuclear genome quantification and characterization of the carrageenophytes *Eucheuma* and *Kappaphycus* (Gigartinales). Journal of Applied Phycology 8: 465-471.

23. Kapraun DF, Bailey JC, Dutcher JA. 1994. Nuclear genome characterization of the carrageenophyte *Hypnea musciformis* (Rhodophyta). Journal of Applied Phycology 6: 712.

24. Kapraun DF, Dutcher JA, Freshwater DW. 1993. Quantification and characterization of nuclear genomes in commercial red seaweeds: Gracilariales and Gelidiales. Hydrobiologia 260/26l: 679-688.

25. Kapraun DF, Dutcher JA, Lopez-Bautista J. 1992. Nuclear genome characterization of the carrageenophyte *Agardhiella subulata* (Rhodophyta). Journal of Applied Phycology 4: 129-137.

26. Kapraun DF, Dutcher JA, Bird KT, Capecchi MF. 1993.
Nuclear genome characterization and carrageenan analysis of *Gymnogongrus griffithsiae* (Rhodophyta) from North Carolina. Journal of Applied Phycology 5: 99 – 107.

27. Kapraun DF, Ganzon-Fortes E., Bird K, Trono G, Breden C. 1994b. Karyology and agar analysis of the agarophyte *Gelidiella acerosa* (Forsskal) Feldmann et Hamel from the Philippines. Journal of Applied Phycology 6: 545-550.

28. Kapraun DF, Lopez-Bautista J., Trono G, Bird KT. 1996. Quantification and characterization of nuclear genomes in commercial red seaweeds (Gracilariales) from the Philippines. Journal of Applied Phycology 8: 125

29. Le Gall Y, Brown S, Marie D, Mejjad M, Kloareg B. 1993. Quantification of nuclear DNA and G-C content in marine macroalgae by flow cytometry of isolated nuclei. Protoplasma 173: 123-132.

30. Lopez-Bautista J, Kapraun DF. 1995. Agar analysis, nuclear genome quantification and characterization of four agarophytes (*Gracilaria*) from the Mexican Gulf Coast. Journal of Applied Phycology 7: 351-357.

31. Magne F. 1964. Recherches caryologiques chez les Floridées (Rhodophycées). Cahiers Biologique Marine 5: 461-671.

32. Maleszka R. 1993. Electrophoretic analysis of the nuclear and organellar genomes in the ultra-small alga *Cyanidioschyzon merolae*. Current Genetics 24: 548-550.

33. Matsuzaki M, Misumi O, Shin-I T, Maruyama S, Takahara M, Miyagishima SY, Mori T, Nishida K, Yagisawa F, Nishida K, Yoshida Y, Nishimura Y, Nakao S, Kobayashi T, Momoyama Y, Higashiyama T, Minoda A, Sano M, Nomoto H, Oishi K, Hayashi H, Ohta F, Nishizaka S, Haga S, Miura S, Morishita T, Kabeya Y, Terasawa K, Suzuki Y, Ishii Y, Asakawa S, Takano H, Ohta N, Kuroiwa H, Tanaka K, Shimizu N, Sugano S, Sato N, Nozaki H, Ogasawara N, Kohara Y, Kuroiwa T. 2004. Genome sequence of the ultrasmall unicellular red alga *Cyanidioschyzon merolae* 10D. Nature 428: 653-657.

34. McLaughlin J, van der Meer JP, Bird NL. 1977. Chromosome numbers of *Gracilaria foliifera* and *Gracilaria* sp. (Rhodohyta) and attempted hybridizations. Journal of the Marine Biological Association United Kingdom 57: 1137-1141.

35. Moreira D, Lopez-Archilla A-J, Amils R, Marin J. 1994. Characterization of two new thermoacidophilic microalgae: genome organization and comparison with *Galdieria sulphuraria*. FEMS Microbiology Letters 122: 109-114.

36. Muravenko OV, Selyakh IO, Kononenko NV, Stadnichuk IN. 2001. Chromosome numbers and nuclear DNA contents in the red microalgae *Cyanidium caldarium* and three *Galdieria* species. European Journal of Phycology 36: 227-232.

37. **Mullahy JH. 1952**. The morphology and cytology of *Lemanea australis* Atk. The Bulletin of the Torrey Botanical Club 79: 471-484.

38. **Necchi O Jr, Sheath RG. 1992**. Karyology of Brazilian species of *Batrachospermum* (Rhodophyta, Batrachospermales). British Phycological Journal 27: 423-427.

39. **Necchi O. Jr, Carmona JJ. 2002**. Somatic meiosis and development of juvenile gametophyte in members of the Batrachospermales sensu lato (Rhodophyta). Phycologia 41: 340-347.

40. Nichols HW. 1964. Culture and developmental morphology of *Compsopogon coeruleus*. American Journal of Botany 51: 180-188.

41. Notoya M, Yabu H. 1979. *Heterosiphonia pulchra* (Okamura) Falkenberg (Ceramiales, Rhodophyta) in culture. Bulletin Faculty of Fisheries, Hokkaido University 30: 129 – 132.

42. Rao CSP, Gujarati AR. 1973. Second meiotic division in the tetrasporangium of *Chondria dasyphylla* (Woodw.) C. Ag. Current Science 42: 361-362.

43. Ravanko O. 1987. Preliminary studies on cells and chromosomes in *Polysiphonia violacea*. Societas pro Fauna et flora Fennica. Flora Fennica. 63: 45-50.

44. Rosenberg T. 1933. Studien über Rhodomelaceen und Dasyaceen. Akadamie Abhandlung.Lund

45. Rueness J, Asen PA. 1982. Field and culture observations on the life history of *Bonnemaisonia asparagoides* (Woodw.) C. Ag. (Rhodophyta) from Norway. Botanica Marina 25: 577-587.

46. Salvador Soler N, Gómez Garreta A, Ribera Siguan MA, Kapraun DF. 2011. Nuclear DNA content variation in life history phases of Bonnemaisoniales (Rhodophyta) from the Spanish Coast. (unpubl. res.).

47. Sheath RG, Cole KM. 1993. Distribution and systematics of *Batrachospermum* (Batrachospermales, Rhodophyta) in North America 2. Chromosome numbers. Phycologia 32: 304-306.

48. Sommerfeld MR, Nichols HW. 1970. Comparative studies in the genus *Porphyridium* Naeg. Journal of Phycology 6: 67-78.

49. Suneson S. 1937. studien über die Entwicklungsgeschichte der Corallinaceen. Kongliga Fysiografiska Soelskapets i Lund. Handlingar 48: 1-101.

50. Suzuki K, Ohta N, Kuroiwa T. 1992. Isolation of the cell-nuclear, mitochondrial and chloroplast DNA from the ultra-small eukaryotic *Cyanidioschyzon merolae*. Protoplasma 171: 80-84.

51. **Svedelius N. 1933**. On the development of *Asparagopsis armata* Harv. and *Bonnemaisonia asparagoides* (Woodw.) Ag. Nova Acta Regiae Societatis Scientiarum Upsaliensis 9: 1-61.

52. Takahashi H, Suzuki K, Ohta N, Suzuki T, Takano H, Kawano S, Kuroiwa T. 1993. An electrophoretic karyotype of *Cyanidioschizon merolae*. Cytologia 58: 477-482.

53. **Thirb HH, Benson-Evans K. 1982**. Cytological studies on *Lemanea fluviatilis* L. in the the river Usk. British Phycological Journal 17: 401-409.

54. Van der Meer JP. 1976. A contribution towards elucidating the life history of *Palmaria palmata* (= *Rhodymenia palmata*). Canadian Journal of Botany 54: 2903-2906.

55. **Webster RN. 1958**. The life history of the freshwater red alga *Tuomeya fluviatilis* Harv. Butler University Botanical studies 13: 141-159.

56. Yabu H, Kawamura K. 1959. Cytological study on some Japanese species of Rhodomelaceae. Memoirs of the Faculty of Fisheries, Hokkaido University 7: 61-72.